TRACE ELEMENTS

in Human and Animal Nutrition

E J UNDERWOOD

Institute of Agriculture

University of Western Australia

Nedlands, Western Australia



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DEDICATION

To my wife and children

for their encouragement

and their forbearance

PREFACE

The real purpose of a preface it has been said, is to break down reader resistance to put the reader in the proper frame of mind to approach the reading of the book Such a purpose has no great appeal to the present author but it may perhaps put the reader in a more sympathetic frame of mind if it is mentioned at the outset that this is a first book not a first book in the author sense but in the subject sense Numerous books have been published on the vitamins but none dealing wholly with the trace elements in human and animal nutrition This is curious when one considers that the history of the trace elements is as stimulating and as romantic as that of the vitamins their economic significance is as great or greater and they lie equally at the root of physiological processes These points will it is hoped become abundantly apparent from the pages which follow It is true that a small book by Stiles called Trace Elements in Plants and Animals" was published in 1946 and one by Monier Williams entitled "Trace Elements in Food" in 1949 but the former devotes only a few pages to animals and the latter is concerned principally with analytical methods and with toxicological and public health aspects of the trace elements rather than with nutrition For this reason the present text can legitimately be regarded as a first attempt to survey the trace elements from the point of view of their nutritional significance to man and his domestic animals

This book is written for those who plan to specialize in nutrition or who are already specialists in nutrition It is not written for those who are primarily dietitians biochemists or pathologists although inevitably there is much in it to interest all three In fact it is impossible to treat the trace elements at all effectively without venturing boldly sometimes even rashly into the biochemistry of the cell on the one hand and into the pharmacology and toxicology of the living body on the other The extent of the movement into either field varies with the element It is governed by no known rationale but rather by the personal interest knowledge and predilections of the author The author's aim has been to present a balanced treatment to explore as fully as space will allow those facets of any element which throw light on the nutritional significance of that element and to exclude arbitrarily other aspects which appear to have no such bearing on nutrition The author has also

thought it important to preserve an historical approach, wherever pos sible It is felt that this has particular value in a first book, not only because it brings together under one cover much interesting material likely to be lost in a welter of original articles and reviews but also because in no other way can the methods and approaches of research be revealed and the step by step development of knowledge be em phasized for the benefit of the young student and researcher

The problem of selection of material—of what to leave in and what to leave out—is always a difficult one but it is especially difficult with the trace elements because of their nature and their ramifications The author has tried to deal thoroughly with all those elements in which naturally occurring deficiencies or excesses are known, because of their obvious economic importance and interest However, all trace elements which have been shown to be essential or which have had nutritional significance ascribed to them have received attention

Complete documentation, with authority for every statement made, was considered to be clumsy and unwarranted Reference is made nevertheless to a very large number of original articles and to most of the excellent reviews which have appeared on individual elements or groups of elements A list of these appears at the end of each chapter Many authors and editors of journals have readily granted permission to reprint various graphs and illustrations all of which are acknowledged in the text The author is indeed grateful for this assistance It is a pleasure to record, also, the help and encouragement received from many friends and colleagues In this connection, special mention should be made of Drs H W Bennetts, D H Curnow, A T Dick R C Rossiter and E G Saint and Messrs A B Beck R J Moir and E Munch Petersen who read various chapters and made many helpful suggestions Any errors of fact or defects in the plan and scope of the book are however the author's own responsibility Finally it is a particular pleasure to record the debt owed to Miss M Keane for her care and skill in the typing of the manuscript and in the laborious task of checking references

E J Underwood

University of Western Australia,
Nedlands Western Australia
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CHAPTER 1

INTRODUCTION

1 The Trace Element Concept

Over the last hundred years and especially in the last thirty years a steadily increasing number of elements has been found to be constantly present in living tissues To a few of them, definite biological functions have been assigned The term trace elements" was quite understandably applied by the early workers to those elements which occur or which function in very small amounts relative to the amounts of the main constituents of the tissues because of the difficulties then associated with measuring the low concentrations involved and their recognition therefore merely as traces" The term minor elements" has also gained some acceptance particularly by plant biologists to describe these elements Both these terms have been quite justifiably criticized as misleading or as having prejudicial implications--the first because it fails to convey the idea of their quantitative significance and the second because it suggests for them a minor or less important role compared with those elements which occur or are required in larger quantities Several alternative names have been suggested The terms "oligo element or "oligo metal (oligo coming from the Greek "oligos" meaning scanty) have been used in the French literature for some years and the terms "micro-element" or "micro nutrient" have the impressive support of several international conferences None of these latter terms, however appears to have made a great deal of headway in spite of their greater descriptive accuracy The name "trace elements" is retained in this book because of its historical associations and its continued general acceptance by most active workers in the field

Nearly every element has been found to occur in living tissues by one method or another at some time or another Spectrochemical methods because of their convenience specificity and accuracy in skilled and critical hands have been particularly useful in the detection and estimation of a series of trace metals in biological materials under a variety of conditions They have in fact played a very important part in giving useful leads in the investigation of several naturally occurring nutritional problems in animals to which reference is made in the appropriate chapters which follow

trace elements and those which cannot be so classed For instance iron is considered a trace metal by some writers and not by others It is certainly required by animals in significantly larger quantities than the other trace elements and it occurs in the body in relatively high concentrations as part of the *hemoglobin molecule On the other hand,* it functions also as a constituent of a number of oxidative enzymes in a manner similar to copper, and is intimately related to copper in its metabolism Moreover in many body fluids and tissues it does not normally occur in concentrations which are as high as those of another acknowledged trace element, zinc For these reasons it is included among the trace elements in this text Even if iron is excluded from the present considerations however, large quantitative differences remain Thus the requirements of mammals for copper are many times those for iodine or cobalt and the concentrations of zinc in animal tissues are many times those of manganese and so on An idea of the magnitude of some of these differences can be gained from an inspection of Table 1, in which typical concentrations of the six essential trace elements in normal human blood are presented

TABLE 1
TYPICAL CONCENTRATIONS[a] OF ESSENTIAL TRACE ELEMENTS IN NORMAL HUMAN BLOOD

Element	Whole blood	Serum
Iron	50 000	80–100
Zinc	700–900	300
Copper	100–120	100–120
Manganese	12–18	4–6
Iodine	8–12	5–6
Cobalt	–	0 8

[a] Measured in μg per 100 ml

Some of the trace elements to which no definite functions have yet been ascribed occur in the blood and tissues in concentrations significantly higher than those of the essential trace elements Bromine and silicon are examples of such elements as is evident from the data given in Chapter 12 Another example of great interest is the alkali metal rubidium Rubidium has been found to occur in most animal and human tissues and organs, including fetal tissues and milk in fairly constant proportions amounting to no less than 20–40 p p m on the dry basis (12) No attempts to find out if these appreciable quantities of this element serve any useful or essential function within the tissues appear to have been made

II The Development of Knowledge of the Trace Elements

Although a great deal of our knowledge of the significance of the trace elements in animal physiology has been accumulated in the last thirty years—in fact since the classical demonstration by E B Hart and his colleagues of the essential nature of copper in mammalian nutrition in 1928 (9)—interest in certain aspects of these elements dates back for over a century The presence of copper in the oxygen transporting hemocyanin of mollusks was first recognized in 1847 (8), iron was shown to be a part of the hemoglobin molecule in 1886 (15), iodine was found by Baumann (1) a few years later to be concentrated in the thyroid—although this element had been used empirically in the treatment of human goiter as early as 1820 (4) zinc was shown to be present in the respiratory pigment of the snail Sycotypus, in 1905 (11) vanadium was demonstrated in the blood pigment of sea squirts in 1911 (10) and the series of early investigations on cell respiration and on iron and oxidative processes which began with Claude Bernard in 1857 (2) pointed the way to the discovery of metalloenzymes and to metal enzyme interactions in catalysis

During this early period also the universal presence of a range of elements in minute quantities in plant and animal tissues and in the soils on which the plants feed was established Many of these early studies are now known to have been quantitatively misleading because the analytical methods then available were rarely equal to the task of estimating accurately the extremely low concentrations involved and because the ease with which contamination can occur was not generally appreciated Nevertheless these "distribution" studies served the very useful purpose of drawing attention to the possible significance of many elements previously disregarded and gave the necessary stimulus to physiological and nutritional investigations designed to ascertain their function if any in living tissues

Interesting and important as these studies were advances in our understanding of the role of the trace elements were exceedingly slow until two different types of investigations began—investigations which bear a striking resemblance in their method of approach to those which have been so successful in illuminating the significance of the vitamins in animal physiology and of the trace elements in plant physiology These are (*1*) the investigation of various naturally occurring diseases of man and animals widely separated geographically which were shown to be due to a dietary deficiency or excess of a particular trace element and (2) the investigation of the effects on animals of highly purified or

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specially constituted diets deliberately designed to have an abnormally low, or high, content of the trace mineral under study.

Outstanding examples of the first type of investigation are (a) the clear demonstration, early in this century, that lack of iodine in the foods and waters of certain regions is the primary cause of endemic goiter of man and farm stock. (b) the revelation, in the 1930's that cobalt deficiency in the soils and pastures of particular areas is the cause of a group of wasting diseases of sheep and cattle, and (c) the demonstration, in the same decade, that excessive intakes of molybdenum from certain pastures are responsible for a severe scouring condition in cattle. These are, of course, considered in some detail in subsequent chapters. The considerable scientific effort which was concentrated upon the solution of these and similar types of "field" problems, and the spectacular results achieved, were very potent factors in the development of a new knowledge about the nutritional significance of the trace elements. A great deal of light was shed upon the ways in which these elements function in the animal body. Soil-plant-animal interrelationships were given added meaning and significance, especially as the soil deficiencies or excesses primarily responsible for the disease conditions in animals sometimes affected plant growth or health as well as plant composition. New and improved methods of estimation of great delicacy and precision were developed in response to the urgent need to trace the distribution of the elements concerned in soils, plants, and animal tissues. The importance of trace element deficiency problems was found to extend far beyond the disease conditions which gave the initial stimulus to the investigations, by the disclosure of the widespread occurrence of more subtle, milder forms of deficiency, less dramatic in their manifestations but often of greater economic importance. Further, the vital significance of a proper balance of minerals in the diet was revealed by the discovery that a number of these dietary disorders resulted not merely from a deficiency or excess of a single trace element, but from a deficiency or excess conditioned by the extent to which other elements, nutrients or special factors are present in the diet.

In the second mode of approach, involving the use of highly purified or specially constituted diets with small laboratory animals, the motivating philosophy was the desire of enquiring minds to learn whether this or that trace mineral serves any useful function in the animal body. Although lacking the stimulus and resources which stem from economic need, these investigations have been equally fruitful and were responsible for the original demonstrations of the essential nature of copper, manganese, and zinc in mammalian nutrition. Many of the early studies

with purified diets especially those of the great French school led by Gabrielle Bertrand and of J S McHargue in Kentucky, during the 1920s, were inconclusive because the diets employed were generally lacking in essential vitamins so that the animals made little growth or survived for only short periods, even when supplemented with the element under study Not until vitamin research had advanced sufficiently to enable diets to be devised low in the element in question but adequate or reasonably adequate in other respects was rapid progress made in this type of study Outstanding in this respect was the Wisconsin school led by the late E B Hart Not only were the researchers of this school extremely successful with purified diets but in the decade following the demonstration of the relation of copper to hemoglobin production in the rat in 1928 they pioneered the use of whole milk or of diets composed largely of milk in trace element studies Milk because of its palatability its richness in many dietary essentials and its natural deficiency in iron copper and manganese has proved to be an invaluable tool to nutrition workers interested in the trace elements

The two approaches to the study of trace elements outlined above were at first distinct and unrelated but they have tended to merge in recent years each contributing to developments in the other in a manner similar to the merging of pure and applied research in the related field of vitamin research Studies of naturally occurring deficiencies or toxicities in man or farm stock have provided new stimuli for the further investigation of the mode of action of trace elements in laboratory animals by disclosing new and often unexpected functions within the body and laboratory studies with small animals have been fruitful in drawing attention to the wider significance of certain elements in field problems previously inexplicable Such cross fertilization in ideas and techniques examples of which are given in the chapters that follow has been a potent factor in increasing our understanding of the nutritional significance of a great number of these elements

Within the last few years a new and powerful weapon the "tracer" isotope has been added to the nutritional physiologist's armamentarium Radioactive isotopes with a suitable half life and a high specific activity are now available for a wide range of elements in amounts and at a cost suited to most biological needs With the trace elements this has proved to be a development of tremendous significance because essential investigations of absorption excretion distribution and combination within the tissues were in some cases impossible and in all cases difficult by conventional methods The value of radioactive isotopes has been shown most convincingly with iodine whose metabolic movements defied

accurate appraisal for many years, and with iron, whose peculiar mechanism of absorption and complex intermediary metabolism have only been revealed by the use of radioactive isotopes of this element With other trace elements, radioactive isotopes have not yet proved of such outstanding value although there is little doubt that they will do so as more research is undertaken with them and as techniques improve

III Mode of Action of the Trace Elements

It was pointed out earlier that the only characteristic which the trace elements have in common is their capacity to function in small quantities It is this capacity which indicates that they must act as catalysts involved in hormone or enzyme systems either as constituent parts of the molecules of hormones vitamins enzymes, or coenzymes or as enzyme activators In fact as Green (7) has said, enzymic catalysis is the only rational explanation of how a trace of some substance can produce profound biological effects" Many examples of trace elements functioning in these ways are now known Iodine has been shown to be an integral part of the thyroid hormone, thyroxine or triiodothyronine, and cobalt of the vitamin, B_{12} iron and copper are known to be irreplaceable components of the molecule of several oxidative enzymes zinc is present in carbonic anhydrase manganese in arginase, and molybdenum in xanthine oxidase and nitrate reductase In addition, many trace minerals have been shown to function as catalysts for a range of more or less purified enzymes *in vitro*

These findings leave little doubt that the trace elements function as activators or as catalysts within the living cell and that they lie at the root of living processes At the same time it is necessary to avoid the rather specious clarity which frequent repetition of such a statement tends to engender The metal ions which include many of the trace elements, appear to act in two different ways in enzyme systems They may be an indispensable part of the protein from which they can only be dissociated with difficulty Examples of such are the iron complexes of the "heme proteins" the cobaltic complex of vitamin B_{12}, and the zinc and molybdenum enzymes mentioned in the preceding paragraph In these cases it would be better, as Williams (13) has suggested to describe the function of the metal ion which is highly specific as one of activation rather than of catalysis of an enzyme reaction The metals may also act in a manner similar to their action in nonenzymatic catalysis In these cases as with the peptidases and the phosphatases, the metal ions are readily dialyzable from the enzymes, which then become somewhat reduced in activity and the catalysis has a relatively low metal

ion specificity In neither case however can a clear exposition of the way in which these elements function yet be given Moreover with the latter group especially most of the studies have been carried out *in vitro* Enzymatic catalysis in these circumstances may not be applicable to *in vivo* enzyme reactions although it is difficult to imagine that they are totally unconnected

In the earlier stages of the trace element era investigators tended to concentrate upon the reaction of the whole organism—upon such gross effects as growth appetite and live weight changes reproductive performance and the like These were obviously necessary and important and are still so but they do not readily illuminate the mode of action of the element in question Subsequent investigations have however moved successively and successfully down the scale of organizational complexity from the whole organism to organs tissues cells cell particulates and finally to individual components of the matrix of enzymes coenzymes and inorganic cofactors within the cell These have proved increasingly revealing in respect to trace element enzyme relationships but, unfortunately, many of the studies of enzymatic changes in the tissues and fluids of living animals suffering from various trace element deficiencies have as yet proved very disappointing Changes in the concentrations or activities of those enzymes or hormones with which the particular trace element *is known to be associated have been demonstrated* under these conditions with some elements notably iron cobalt copper and manganese But frequently marked retardation in growth and profound clinical changes have been observed in the deficient animal well before any reduction in the amount or activity of the enzyme or enzymes concerned can be detected This is particularly outstanding with zinc as is shown in Chapter 7 These findings do not of course denigrate the trace element enzyme relationship but they indicate that *the functions of these elements* in living systems extend far beyond our present understanding and that these functions are more subtle and more complex than can be appreciated at the present state of knowledge

Whatever future research may hold in this respect it nevertheless seems clear that the trace elements constitute such a small proportion of the structural make up of plants and animals that they must function as catalysts Bertrand has put forward the interesting hypothesis that they have played a significant part in evolution by increasing the number of chemical reactions within the cell and the consequent elaboration of morphological structures physiological functions and biochemical potentialities In more emotive language he suggests that the living organism both plant and animal is "a kind of oligarchy in which the masses

composed of the more passive elements, are governed by a minority consisting of the catalytic [trace] elements (3)

The specificity of living cells in their mineral requirements is remarkable Neither similar physical nor similar chemical properties imply similar physiological properties Even cobalt and nickel which have closely similar atomic weights and ionic radii and a host of very similar physical and chemical characteristics are apparently completely differentiated by living organisms There is no satisfactory evidence that nickel can either replace cobalt in ruminant metabolism or effectively economize in its utilization or that it can stimulate erythropoiesis as cobalt does A similar position exists, as far as functional activity is concerned, in relation to barium and strontium and to iodine and bromine although in this latter case there are indications that the thyroid gland will concentrate bromine in its cells under conditions of low iodine intake

IV Trace Element Interactions

The possibilities of significant interactions among the trace elements ve not received the attention in animal physiology, which they un ubtedly deserve in spite of the considerable body of knowledge dicating the importance of such interactions in the nutrition of plants ome studies have been undertaken on the effects of one element in counteracting the toxic effects of excessive intakes of another such as arsenic in selenium toxicity and copper in zinc and molybdenum toxicity, and on the influence of comparatively massive intakes of one element on the absorption of another, such as phosphate on iron and manganese uptake Until recently however little evidence has been presented to suggest that there is an interrelationship between the amounts of the trace elements required by animals or in their metabolism within the body at physiological levels

The earliest of such physiological interactions to be revealed was that between iron and copper Both these elements are concerned as one of their functions, with hematopoiesis so that a deficiency of one will clearly limit the requirement of the other It is usually stated that copper is necessary for iron utilization in blood formation—a statement which can be substantiated by abundant evidence—but iron must also be necessary for copper utilization at least to the extent that copper is concerned in hematopoiesis Trace element interactions have even more strikingly been demonstrated by recent studies which have disclosed a reciprocal antagonism between copper and molybdenum It has been shown further by Dick (5) that the inhibiting effect of molybdenum on copper retention is markedly influenced by the level of inorganic sulfate

intake of the animal and that molybdenum excretion in the sheep is profoundly affected also by the inorganic sulfate level of the diet

These findings are discussed more fully in the chapters dealing with copper molybdenum and zinc, but it is clear that studies of copper metabolism and copper requirements are of little value unless the molybdenum and inorganic sulfate levels in the diet are known and that studies of molybdenum metabolism especially of its retention and excretion are largely worthless unless the copper and inorganic sulfate contents of the diet are known How far these particular relationships apply to nonruminant species remains to be determined but evidence has already been obtained that the copper requirement of the rat is greatly affected by the level of dietary zinc It seems reasonable to expect that other trace element and nutrient interactions of this type exist In any case it is obvious that considerable caution should be exercised in the interpretation of nutritional and metabolic studies with the trace elements in which attention is restricted to a single element

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largely to variations in the amounts of iron stored in the liver and in the levels of hemoglobin in the blood Thus the pig, which has relatively little iron in its body at birth, has a low liver iron at this time and no "polycythemia of the newborn Whereas the rabbit, which has a very high body iron at birth (Table 3), is born with exceptionally large stores of iron in its liver (132) These liver iron stores in the rabbit are four times as high as those of the newborn of any other species studied and may amount to more than one third of the total iron in the newborn rabbit s body

TABLE 3

IRON CONTENTS[a] OF BODIES OF DIFFERENT SPECIES[a] (132)

	Species						
	Human	Pig	Cat	Rabbit	Guinea pig	Rat	Mouse
Adult	74	90	60	60	—	60	—
Newborn	94	29	55	135	67	59	66

[a] Measured in Fe p p m of fat free tissue

Sex has an important bearing on the accumulation of iron in the body of some species but the effect is not consistent in all species Women have significantly lower levels of circulating hemoglobin and serum iron than men, which, in view of the high proportion of body iron present as blood hemoglobin suggests a lower total body iron in the female sex Female rats on the other hand have a higher total body iron than males and accumulate more iron in their livers on the same dietary regime (93) Female mice and poultry also carry significantly greater concentrations of iron in their livers than do males, although no such sex difference is apparent in rabbits or guinea pigs (131) There appear to be no data on this point for the larger farm animals

III Iron in Blood

1 *Hemoglobin*

a *Structure* Hemoglobin can be defined as a compound of a ferrous iron porphyrin (heme) with a protein (globin) which forms with oxygen a reversible molecular compound with a red color and a definite type of absorption spectrum The heme component seems to be identical in all species Its chemical structure was established as ferroprotoporphyrin 9, type III, and its synthesis accomplished in 1929 (35) The protein portion of the hemoglobin molecule differs from species to species and even from embryo to adult in an individual The protein (globin) differences are shown by slight differences in spectral absorption, oxygen

affinity crystalline form, and amino acid composition All mammalian hemoglobins contain four hemes to one globin that is, four atoms of iron to each hemoglobin molecule but invertebrate hemoglobins may contain very many more hemes to each globin unit Union between the heme and globin *molecules is believed to be brought about by electro* static attraction between ionized propionic acid groups and surface basic groups of heme and by coordination of iron with a nitrogen containing group of the globin probably imidazole from histidine This latter union stabilizes the iron in the ferrous condition and allows it to be reversibly bonded to oxygen thus permitting hemoglobin to function as an oxygen carrier (97)

The hemoglobin molecule is similar in size in all mammals birds and fishes It has a molecular weight of 68 000 and an iron content very close to 0 34% Pure hemoglobin obtained from the blood of different mammalian species has been reported to contain 0 30–0 59% iron, depending upon the species but it is probable that the higher values are inexact, due to difficulties in the preparation of the hemoglobin as well as in the determination of the iron Small species differences in iron content no doubt exist, consequent upon variations in the nature of the globin component of the molecule but satisfactory values characteristic of particular species cannot yet be given

b *Formation* The exact steps by which hemoglobin synthesis is accomplished in the bone marrow are far from clear Globin is apparently first built up in the differentiating stem cell and synthesis of heme and its attachment to globin take place in the later stages of red cell development. Definite evidence that glycine and acetate are utilized by the body as starting compounds in heme production is now available (10) It seems probable that these are first condensed into pyrrole ring units four of which unite to give protoporphyrin into which the iron enters to form heme Protoporphyrin is normally present in the erythrocytes and particularly in the reticulocytes but not in blood plasma It has been identified in the *megaloblasts and the erythroblasts* of fetal bone marrow (100) Also an increased protoporphyrin content of the bone marrow and of the erythrocytes following hemorrhagic anemia has been reported in rabbits (65) Iron has been detected histochemically in the nuclei of the developing red cell and found to occur in considerable quantities in bone marrow The incorporation of this iron into the protoporphyrin to form heme and the combination of the heme *and the globin to form hemoglobin seem to be localized* in the erythrogenic tissue within the developing red cell Copper is necessary at some point or points within this process but the exact stage at which

it exerts its action is still not known This question is considered more fully in the chapter on copper

The extent to which factors influencing hemoglobin formation and red cell maturation can be independent of each other is also far from clear although they must be closely correlated processes If for instance erythrocytes cannot mature without at least a partial complement of hemoglobin, which seems highly probable, factors such as a deficiency of iron, which limit hemoglobin synthesis, will also limit erythrocyte production Similarly, factors such as vitamin B_{12} which are known to affect red cell maturation, will also affect hemoglobin formation because hemoglobin is confined to the red cells and there is a limit to the hemoglobin content of the individual cell

c *Levels in Blood* The concentration of hemoglobin in the blood is of the same order in all mammalian species. The normal range of values found for apparently healthy adults of different species is too large to give much meaning to arbitrary single figures but the following normal values can be taken to represent the real species differences which undoubtedly exist men 15–16 women, 13–14 rat 14–15 dog 13–14 cow, pig and rabbit, 11–12 and sheep, goat and horse 10–11 g hemoglobin per 100 ml of blood The *total* hemoglobin in the body of mammals is however directly proportional to body weight The mean of the five species examined by Drabkin (25) was 12 7 g hemoglobin per kg body weight

In man the concentration varies with age sex, nutrition pregnancy state of health environment (climatic and barometric), and perhaps race Several modern studies of hemoglobin levels in human populations have been carried out (86 88 128) which indicate the extent of normal variability and illustrate the influence of age and sex (Fig 1) High levels at birth are characteristic of most but not all mammals The blood of the newborn human contains as much as 20–22 g hemoglobin per 100 ml Under ordinary conditions of feeding this level falls rapidly to about 12 g per 100 ml at 3–4 months at which level it usually remains or falls slightly until the baby is about a year old when a slow rise to adult values begins There is a striking rise in the hemoglobin level of the blood of males at puberty compared with that of the female The higher values of the male continue throughout the life span (Fig 1) Whether this sex difference reflects subtle metabolic differences attributable to hormonal diversities between the sexes or whether the female is in fact, usually slightly anemic due to menstrual blood losses and consequent depletion of iron stores is still being debated The bulk of evidence however, is against the latter explanation It will be shown

later that the loss of iron in the menstrual blood imposes an appreciable additional requirement for dietary iron which can be met with difficulty if at all from many diets and that some success has been obtained in increasing the mean hemoglobin levels of women by the administration of iron (130) On the other hand many attempts to raise hemoglobin

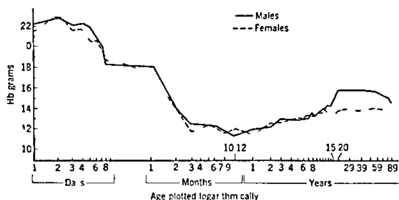


Fig 1 Hemoglobin values in g per 100 ml blood at different ages throughout life The rapid fall in both sexes in early life and the rapid rise in males following puberty are clearly shown (Walsh *et al* 128)

levels in women by such means including a very recent critical study by Garry *et al* (39a) have been completely unsuccessful and most convincing of all there is no significant tendency for the hemoglobin levels of the blood of females to rise to those of males after the menopause or after hysterectomy when these blood losses no longer occur (124)

Really critical data for other species on sex differences of this kind in which all the conditions known to influence hemoglobin concentrations are controlled are surprisingly few Examination of the heterogeneous mass of data which does exist gives little support to any generalization about higher levels for males than females in mammals An extensive study in the bovine revealed a small but significant difference between breeds but no significant sex differences within breeds (15) The mean of 98 samples from 30 bulls was 11 2 g hemoglobin per 100 ml and that of 916 samples from 498 cows was 11 1 g per 100 ml A recent study with normal rats disclosed no significant sex difference in hemoglobin values and no significant changes following castration in either males or females (61)

Evidence that the small fall in mean hemoglobin level which occurs during pregnancy in women is a phenomenon common to mammals is lacking although no such fall takes place in pregnant ewes (78) Thus

fall during pregnancy in women has been observed by many workers and has been associated with a true hydremia the degree of which varies within wide limits (23) The extent of the fall in hemoglobin values and its development during pregnancy are illustrated in Tab with data taken from an Australian survey (128)

TABLE 4

MEAN HEMOGLOBIN VALUES AT VARIOUS STAGES OF PREGNANCY[a] (128)

Weeks of pregnancy	Number of women	Mean	Standard deviation
8–12	144	13.26 ± 0.087	1.00 ± 0.059
12–16	116	12.71 ± 0.087	0.88 ± 0.058
16–20	108	12.62 ± 0.098	1.01 ± 0.068
20–24	118	12.34 ± 0.087	0.94 ± 0.061
24–28	124	12.36 ± 0.073	0.81 ± 0.052
28–32	155	12.10 ± 0.084	1.05 ± 0.059
32–36	171	12.19 ± 0.078	1.02 ± 0.055
36–40	107	12.23 ± 0.108	1.13 ± 0.077

[a] Mean of all nonpregnant women in survey was 13.9 g per 100 ml

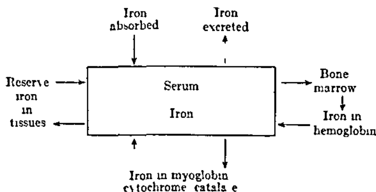
How far these reduced hemoglobin levels of pregnancy represe what may be called a physiological anemia rather than a true anemi consequent upon the extra iron demands of the fetus can only be determined in any individual by a complete hematological investiga tion In fact, if hemoglobin levels 25% or more below normal are taken as suggested later as the criteria of an anemic condition it can hardly be described as an anemia at all Nevertheless several workers have been successful in preventing the decrease in hemoglobin values by administering therapeutic doses of iron during pregnancy (133) These findings together with the probability that the fetus has first demand on the mother's iron stores and the indirect evidence of iron deficiency provided by the existence of low serum iron values and increased iron binding capacity of the serum during pregnancy conspire to suggest strongly that in most cases this condition is a low grade iron deficiency anemia and that the majority of pregnant women would benefit from iron therapy

The maintenance of the level of circulating hemoglobin is an active metabolic process involving considerable daily production of hemoglobin even in adult males free from chronic or accidental blood loss Thus it to replace the regular destruction of erythrocytes which takes place in the cells of the reticulo endothelial system The average life of the erythrocyte in man is about 125 days (51) Since the total mass of

circulating hemoglobin approximates 900 g in a 70 kg man 7 2 g of hemoglobin (900/125) is broken down daily, liberating 24 4 mg ($7 2 \times 3 5$) of iron Most of this iron is conserved that is it does not leave the body, and is available for the replacement of the destroyed hemoglobin This amount of endogenous iron metabolized daily far transcends the amounts of iron absorbed daily from ordinary diets and indicates the extent to which endogenous sources dominate the intermediary metabolism of iron

2 *Serum Iron*

About thirty years ago the constant presence of nonhemoglobin iron in blood serum was definitely established (36) Until this time the small quantities of iron occurring in serum were thought to originate from slight hemolysis of the red cells Subsequent investigations established the fact that the iron of serum does not occur in the free state but is completely bound to a specific protein known as transferrin (57) or siderophilin (99) the sole function of which appears to be the transport of iron from one site in the body to another The total iron in the plasma of a normal adult is about 3 mg (Table 2) The daily turnover of iron in normal hemoglobin destruction and new formation amounts to about 24 mg which is very much larger than the amount ordinarily absorbed from the daily diet As was indicated in the preceding paragraph changes in the rate of hemoglobin destruction or synthesis are therefore likely to be of far greater quantitative significance in determining serum iron levels than are changes in rates of absorption The key position of serum iron in intermediary iron metabolism and the ways in which its level can be influenced by the relative rates at which the different processes involving iron are taking place are illustrated diagrammatically below



The role of serum iron in iron metabolism (Kaldor 69)

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Weeks of pregnancy	Number of women	Mean	Standard deviation
8–12	144	13 26 ± 0 087	1 00 ± 0 059
12–16	116	12 71 ± 0 087	0 88 ± 0 058
16–20	108	12 62 ± 0 098	1 01 ± 0 068
20–24	118	12 34 ± 0 087	0 94 ± 0 061
24–28	124	12 36 ± 0 073	0 81 ± 0 052
28–32	155	12 10 ± 0 084	1 05 ± 0 059
32–36	171	12 19 ± 0 078	1 02 ± 0 055
36–40	107	12 23 ± 0 108	1 13 ± 0 077

[a] Mean of all nonpregnant women in survey was 13 9 g per 100 ml

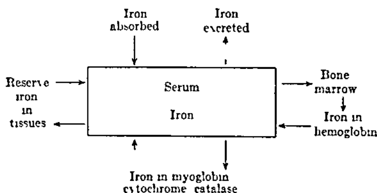
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circulating hemoglobin approximates 900 g in a 70 kg man, 7.2 g of hemoglobin (900/125) is broken down daily, liberating 24.4 mg (7.2×3.5) of iron. Most of this iron is conserved, that is, it does not leave the body, and is available for the replacement of the destroyed hemoglobin. This amount of endogenous iron metabolized daily far transcends the amounts of iron absorbed daily from ordinary diets and indicates the extent to which endogenous sources dominate the intermediary metabolism of iron.

2. *Serum Iron*

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The role of serum iron in iron metabolism (Kaldor, 69)

Siderophilin has been isolated crystallized and characterized as a salmon pink β_1 globulin occurring in Fraction IV 7 (Cohn), with a molecular weight of 90 000 (98) Each molecule is capable of binding two atoms of iron which are in the ferric state and can be removed from the complex either when converted to the ferrous state by reducing agents or by dialysis at pH 5 or less (98) Present evidence suggests that siderophilin serves as a true carrier for iron in the same way that hemoglobin acts as a carrier for oxygen (67) It is probable therefore that iron leaves the blood stream in an ionized form and not as an iron globulin complex but the manner in which iron is made available from its combination with siderophilin to take part in other functions is still a subject of dispute It has been suggested that a small fraction of iron existing in ferrous ionic form in equilibrium with siderophilin accounts for the transfer across membranes (67) whereas Flexner has proposed that a more complex enzymatic factor involving ferritin rather than simple diffusion accounts for such transfer (32)

Since the iron binding property of siderophilin is specific to this protein and since iron cannot exist in serum for any length of time in the free state it follows that the capacity of serum to transport iron is governed by its content of siderophilin Normally only a portion of this siderophilin carries iron In the healthy human subject the proportion is about one third the remaining two thirds being known as the latent iron binding capacity of the serum Intravenous injection of iron produces a rise to the saturation value only that is to the iron binding capacity of the siderophilin present Any excess iron rapidly leaves the blood stream to enter the tissues where toxic effects may ensue (56) There are, therefore, four values of significance in iron transport These are serum iron (SI) the latent iron binding capacity (LIBC), the total iron binding capacity (TIBC) which is the sum of the SI and the LIBC and the percentage saturation of the iron binding protein (PS), which is defined as SI divided by TIBC expressed as a percentage Each of these values has been shown in the human subject to vary characteristically for certain physiological and pathological conditions (69) The nature and the extent of the variations are illustrated in Table 5

Values for normal human subjects in different environments have been obtained by a number of investigators (69) These show fair agreement particularly as many of them were obtained before the importance of the diurnal rhythm (see below) in serum iron was properly appreciated The total iron binding capacity varies on the average from about 300–350 μg per 100 ml of serum the serum iron from 90–120 μg per 100 ml and the percentage saturation from 30–40% Many individual values for ap

SERUM IRON TOTAL IRON BINDING CAPACITY AND PERCENTAGE SATURATION IN VARIOUS CONDITIONS

Subject and condition	Number of cases	Serum iron (μg per 100 ml)	Total iron binding capacity (μg per 100 ml)	Saturation (%)	Source (see ref 69)
Normal adults–male	15	106(87–147)	311(251–432)	34(30–44)	Rath and Finch
Normal adults–female	15	94(72–130)	288(224–414)	33(22–44)	Rath and Finch
Normal adults–male	15	127(79–196)	347(306–396)	36(24–49)	Cartwright and Wintrobe
Normal adults–female	15	123(101–164)	371(316–429)	33(26–42)	Cartwright and Wintrobe
Normal adults–male	?	135(75–195)	–	(26–58)	Kaldor
Normal adults–female	?	110(44–176)	–	(18–54)	Kaldor
Normal adults–male and female	27	115 ± 42	–	–	Lahey *et al*
Normal adults–male	20	–	311 ± 28	39	Bendstrup
Normal adults–female	20	–	309 ± 27		
Adult–iron deficiency anemia	10	29	346	9	Rath and Finch
Adult–iron deficiency anemia	6	23(8–49)	333–500	6(2–12)	Kaldor
Adult–iron deficiency anemia	9	26 ± 7	–	–	Lahey *et al*
Adult–hemochromatosis	9	224	247	91	Rath and Finch
Adult–hemochromatosis	14	234 ± 62	–	–	Lahey *et al*
Adult–hemochromatosis	9	261(130–318)	261(130–348)	100	Kaldor
Adult–pregnant	30	91 ± 38	–	–	Lahey *et al*
Adult–aplastic anemia	8	203(103–272)	–	–	Lahey *et al*
Adult–pernicious anemia	9	173 ± 55	–	–	Lahey *et al*
Adult–infection	37	57 ± 28	–	–	Lahey *et al*
Adult–infection	10	44	220	20	Rath and Finch
Infants–iron deficiency anemia before treatment	12	31	404	8	Lahey *et al*
Infants–iron deficiency anemia after treatment	12	78	352	20	Lahey *et al*
Adults–transfusion hemosiderosis	4	260	260	100	Rath and Finch

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parently healthy subjects, however lie well outside these average normal ranges There is no significant difference between the sexes in total iron binding capacity but women exhibit significantly lower serum iron levels, and hence lower percentage saturation values than men, the difference being of the same order as the sex difference in hemoglobin and red cell concentrations

In addition to variation occurring in the individual from day to day and between individuals at the same time on the same day a well marked diurnal variation in serum iron exists This was first shown by Vahlquist (125) who found 15 normal male subjects to have a mean serum iron of 135 ± 10 6 μg per 100 ml at 8 a m and of 99 ± 9 2 μg per 100 ml at 6 p m A regular diurnal rhythm of this order, or somewhat smaller, characterized by high morning and low evening values, has since been confirmed by several groups of investigators The magnitude of the diurnal variation is greatly diminished in various erythropoietic disorders, including those characterized by decreased and by increased erythropoiesis (48) In night workers the diurnal variation is also somewhat diminished but is reversed to higher evening than morning values (48)

No completely satisfactory explanation of the diurnal rhythm in serum iron is yet available but light has been shed on the problem by the recent finding of Laurell (68) that both the maximum and minimum diurnal levels of serum iron coincide chronologically with the corresponding levels of the likewise rhythmically varying serum bilirubin concentrations As a considerable part of serum iron and of serum bilirubin is normally derived from hemoglobin released during the destruction of red cells it can be supposed that the diurnal variation in serum iron and bilirubin is ascribable to variations in the different phases of hemoglobin metabolism It suggests further that the rate of destruction of hemoglobin is slightly greater during sleep than during hours of activity

It is apparent from the figures given in Table 5 that serum iron levels are well below normal in iron deficiency anemia and in infections and are slightly below normal in pregnancy The first condition is accompanied by an increase in the iron binding protein of the serum to levels above normal, giving extremely low percentage saturation values whereas in infection the percentage saturation is also low but the iron binding protein is significantly reduced In hemochromatosis transfusional hemosiderosis aplastic anemia and pernicious anemia on the other hand, serum iron is two to three times the normal level and the iron binding protein of the serum is fully saturated but slightly below normal concentration

The iron of serum can be conceived as a pool into which iron at varying rates enters leaves and is returned for the synthesis and resynthesis of hemoglobin and the other iron containing complexes This iron is in dynamic equilibrium with the various processes involved in the addition of iron to and the withdrawal of iron from the serum If the rate at which iron leaves the plasma exceeds that at which it can be delivered to the plasma the level will obviously fall and vice versa Thus in iron deficiency low serum iron values and percentage saturation are explainable on the basis of low intake depletion of body iron stores and reduced hemoglobin destruction accompanying the anemia and in infection on the basis of the increased affinity of the tissue storage depots for iron In hemochromatosis high serum iron values and saturation of the iron binding protein are associated with excessive absorption of iron and deposition throughout the body and in aplastic anemia and pernicious anemia with increased absorption and a bone marrow block to hemoglobin synthesis in the presence of adequate body iron stores

The variations in iron binding protein levels are not so readily explained It is tempting to argue that increases or decreases in total iron binding capacity parallel the need of the body for greater or lesser transport of iron and that the degree of saturation regulates iron transport Such a hypothesis is not fully acceptable It has been shown that in animals on diets which allow excessive iron absorption the iron binding protein becomes fully saturated after about two weeks yet iron absorption continues fully as rapidly over the next three to four weeks (69) Moreover injections of iron binding protein exert only a very temporary effect on the serum iron level On present evidence it appears that the carrier protein of the serum plays a merely passive role in iron transport Whether it occurs and functions similarly in all mammals is not known

Very little information is available on serum iron or iron binding capacity in species other than the human It is apparent however that the position in the rat differs in several important respects from that of man (61) Both serum iron and total iron binding capacity are appreciably higher in normal rats than in man and the sex difference is reversed The serum iron values of normal female rats have been shown to be 50% higher than those of normal males and following ovariectomy to decline to values approximating those of normal males Castration of the males produced no significant changes A decline in serum iron with age was observed in both normal males and females and was arrested by castration (61) These interesting findings warrant further study and extension to other species

the sinuses and villi of this tissue and to increase within the kidney tubule cells after hemoglobin leakage through the glomeruli (40)

These findings leave no doubt that ferritin is an important form of storage iron but recent investigations by Shoden and co workers (106) indicate that this function of ferritin is intimately shared with hemo siderin Radioiron administered orally or parenterally to rabbits was found to be stored in liver and spleen as hemosiderin and also as ferritin the proportion depending upon the level of the dose and the iron status of the tissues (106) At physiological levels of tissue iron there was a slight preponderance of ferritin over hemosiderin iron but with increasing concentrations of iron, hemosiderin stores predominated At high levels additional storage iron was reflected in a quantitative increase in hemosiderin in both human and rabbit tissues These workers showed further that when mobilization of iron occurs it is derived from both ferritin and hemosiderin fractions of the tissue It is apparent, therefore that these two forms of storage iron are intimately associated and functionally related

Ferritin has acquired further physiological significance by the demon stration that it or rather SH ferritin will produce an antidiuretic re sponse (4) and by its identification with a vasopressor principle in the liver which is concerned with the regulation of peripheral circula tion (84) Limited studies of ferritin in certain disease conditions of man (5 41) have also provided some interesting facts which cannot yet be evaluated It is apparent that a very great deal remains to be learned of this compound, both in health and disease before its signifi cance to the body can be fully appreciated In this connection it may be mentioned that attempts to synthesize ferritin from apoferritin and an iron compound have not so far been successful the factors influenc ing the incorporation of iron into apoferritin are unknown apoferritin has not been demonstrated in any of the tissues concerned with ferritin production and ferritin itself has not yet been shown to be present in the tissues of ruminants or other farm stock

2 *Hemosiderin*

Historically hemosiderin has been regarded as the main storage form of iron It has become displaced from its former prominence by the more fashionable ferritin but it is becoming increasingly apparent as was indicated when discussing ferritin that the two forms of iron are intimately related in both form and function It is not unlikely in fact, that these compounds differ only in physical form and that a graded series of molecular aggregates exist from ferritin to the visible particles

of hemosiderin (106) Certainly iron in the tissues can pass readily from one form into another

Hemosiderin is a colloidal ferric hydroxide phosphate of unknown constitution which exists in the tissues as a brownish yellow granular pigment the iron in which is microscopically stainable As Josephs (59) has put it "hemosiderin is the form in which iron of the tissues becomes visualized This contrasts with the iron in ferritin which can not be distinguished histochemically in this way The iron in hemosiderin may be as high as 35% by weight and has similar magnetic properties to that in ferritin According to Granick (40), hemosiderin represents tissue iron in excess of the binding capacity of apoferritin and the formation of hemosiderin is viewed as resulting from the deposition of iron in the tissues at a greater rate than the appropriate amount of apo ferritin can be synthesized In consequence the ferric hydroxide phos phate units polymerize into large stainable granules (63) The findings of Shoden and his collaborators (106) previously mentioned do not invalidate this hypothesis but they suggest that it is an oversimplification

Excess deposits of iron as hemosiderin occur in the tissues in various pathological conditions including hemochromatosis and transfusional hemosiderosis in man The aggregates may be so large as to interfere with cell function and impair iron mobilization but the point at which such overload induces pathological changes cannot yet be defined These deposits are especially large in the liver and spleen but other tissues including the myocardium pancreas and adrenal cortex are also fre quently involved All these organs may contain 50 to 100 times the normal amount of iron chiefly as hemosiderin (25) Similar large deposits of hemosiderin have been found in the liver kidney and especially the spleen of sheep suffering from cobalt (119) and copper (89) deficiency

It is important to realize that hemosiderin exists in small amounts in the bone marrow of normal individuals and can be mobilized for hemo globin synthesis (96 111) Histochemical examination of aspirated samples of bone marrow for hemosiderin has in fact been shown to be a reliable index of iron stores and may be the decisive test in diagnosing anemia due to iron deficiency and in differentiating between this type of anemia and that due to infection (111) In the develop ment of iron deficiency anemia the earliest change is the contraction of the iron reserve and in patients with such anemia the stainable marrow iron (hemosiderin) is either absent or present in only minute amounts In those with anemia associated with infection there is usually an increase in marrow (hemosiderin) iron (111)

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These findings leave no doubt that ferritin is an important form of storage iron, but recent investigations by Shoden and co workers (106) indicate that this function of ferritin is intimately shared with hemosiderin Radioiron, administered orally or parenterally to rabbits was found to be stored in liver and spleen as hemosiderin and also as ferritin, the proportion depending upon the level of the dose and the iron status of the tissues (106) At physiological levels of tissue iron there was a slight preponderance of ferritin over hemosiderin iron but, with increasing concentrations of iron, hemosiderin stores predominated At high levels additional storage iron was reflected in a quantitative increase in hemosiderin in both human and rabbit tissues These workers showed further that when mobilization of iron occurs it is derived from both ferritin and hemosiderin fractions of the tissue It is apparent therefore that these two forms of storage iron are intimately associated and functionally related

Ferritin has acquired further physiological significance by the demonstration that it or rather SH ferritin will produce an antidiuretic response (4) and by its identification with a vasopressor principle in the liver which is concerned with the regulation of peripheral circulation (84) Limited studies of ferritin in certain disease conditions of man (5, 41) have also provided some interesting facts which cannot yet be evaluated It is apparent that a very great deal remains to be learned of this compound both in health and disease, before its significance to the body can be fully appreciated In this connection it may be mentioned that attempts to synthesize ferritin from apoferritin and an iron compound have not so far been successful the factors influencing the incorporation of iron into apoferritin are unknown apoferritin has not been demonstrated in any of the tissues concerned with ferritin production and ferritin itself has not yet been shown to be present in the tissues of ruminants or other farm stock

2 *Hemosiderin*

Historically hemosiderin has been regarded as the main storage form of iron It has become displaced from its former prominence by the more fashionable ferritin but it is becoming increasingly apparent as was indicated when discussing ferritin that the two forms of iron are intimately related in both form and function It is not unlikely, in fact that these compounds differ only in physical form and that a graded series of molecular aggregates exist, from ferritin to the visible particles

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3 *The Iron Content of the Liver and other Organs*

A vast amount of miscellaneous data exists on the total iron and the iron concentration of most of the organs of the body of a fairly wide range of animal species Data for the liver exceed those of other organs because of the early recognition of its special responsibility as the main site for the storage of iron and the value of such data therefore as an index of the iron status of the animal. A further stimulus to investigation of the iron content of different organs came from the need for assessing the relative values of these organs as sources of iron in human dietaries

Any synthesis of the data into a comprehensible form is complicated by the fact that some of the results were obtained on perfused tissues and some on tissues which retained variable amounts of blood with its high content of hemoglobin iron With some organs such as the spleen this is obviously extremely important because of the large amounts of blood retained whereas with others such as skeletal muscle the difference between perfused, or washed and nonperfused samples is very small (62) Moreover not only does the amount of blood retained by tissues vary but the hemoglobin, and hence the iron content of this blood may also vary owing to dietary or other causes It is unfortunate that so few workers have followed the excellent example set by Zaleski (134) as long ago as 1886 Zaleski studied the iron content of the livers of a variety of animals following perfusion In recent years however this problem has been overcome by the tendency of some workers to measure the inorganic or nonhemin iron, as well as the total iron of various organs

In spite of this confusion the following generalizations appear permissible (a) The concentrations of iron in the different organs and tissues of adults show no characteristic species differences, that is the between species variation is in general no greater than the within species variation (b) The spleen and the liver in that order contain the highest concentrations of both total and nonhematin iron followed, usually at some distance by such tissues as the kidneys heart, skeletal muscles pancreas and brain which generally contain only one half to one tenth of the concentrations in the liver or spleen The liver because of its size contains the largest total amount of iron (c) The iron content of the liver spleen and bone marrow is very greatly reduced in iron deficiency and hemorrhagic anemia but the reduction in other tissues, notably the skeletal muscles kidney pancreas and brain is much smaller In all cases the reduction is to a base line below which tissues will not give up iron under any conditions of stress Mean figures

illustrating some of these points and providing an idea of the actual magnitude of the various iron concentrations in tissues are presented in Table 6

In some species such as the rat rabbit sheep and man there appears to be almost no limit to the storage capacity of the liver for iron as the result of iron feeding or copper deficiency or infection but in the dog it is apparently much more difficult to raise liver iron by feeding which indicates a possible species difference Enormous increases in the iron content of livers in cases of human malignancy and chronic infection up to a total of 10 g in some cases have been reported (42) Some of these findings have already been referred to in the section dealing with hemosiderin

4 *Iron in Milk*

A very large number of highly variable values for the iron content of milk especially cow s milk has been recorded Some of this variability is associated with faulty analytical methods particularly in the case of many of the early values which are now known to have been much too high Insufficient care in avoiding contamination may also have been a factor in some cases Contamination with iron from metal receptacles readily occurs in the handling and processing of milk often more than doubling its iron content (31 58) Appreciable individual variation exists however in milk taken directly from the animal The iron content of milk varies also with species and with stage of lactation within species but it appears to be independent of the iron intake of the lactating animal Administration of iron salts to cows sows and women has repeatedly been shown to be quite ineffective in raising the iron content of their milk to levels above normal This is similar to the position with copper but whether under conditions of iron deficiency the iron content of the milk is reduced below normal as the copper content of the milk is reduced in copper deficiency is not known The inability of animals given dietary additions of iron to secrete milk with an iron concentration above levels which are normal for the species contrasts greatly with the position for zinc manganese cobalt and iodine where as will be shown later very substantial increases in the levels present in milk can be achieved by such means

Milk with the exception of that of the rat is an extremely poor source of iron compared with almost every other food normal to mammals Cow s goat s and human milk taken in full lactation are very similar in iron content A high proportion of the most acceptable values falls between 0 3 and 0 6 mg Fe per liter but many individual samples lie

TABLE 6
THE MEAN CONCENTRATION[a] OF IRON IN VARIOUS TISSUES AND ORGANS OF ADULT MAMMALS

	Spleen	Liver	Kidney	Bone	Heart muscle	Skeletal muscle	Pancreas	Brain	Authority
Normal dogs (perfused)	46 5	24 6	4 6	15 0	3 9	3 7	2 1	—	Hahn and Whipple (47)
Anemic dogs (perfused)	6 6	4 0	2 9	3 5	2 5	3 3	1 1	—	
Normal bovine (not perfused)	8 9	8 0	5 5	—	4 8	3 9	6 0	2 3	Elvehjem and Peterson (29)
Normal rabbit (not perfused)	33 6	13 2	6 3	13 7	9 9	2 0	—	10 9	
Normal ovine (not perfused)	67 0	14 0	12 0	—	—	—	—	—	Underwood (119)
Normal human[b] (not perfused)	11 5	6 9	0 7	12 7	—	—	0 9	0 9	Tompsett (117)

[a] Measured in mg of iron per 100 g of fresh tissue

[b] Nonhematin iron only It should be noted that all tissues contain considerable quantities of hematin iron According to cFarlane (79) approximately one half of the iron in perfused rat liver tissue is in nonhematin form Few data on this aspect iron are available for other organs and species

outside this range. Cow's colostrum contains 3 to 5 times these concentrations of iron. In a recent careful study, human milk was found to average 0.5 mg Fe per liter and cow's milk 0.45 mg Fe per liter (31). Sow's milk, on the other hand, is slightly richer in iron. Venn, McCance and Widdowson (126) give values ranging from 1.4–2.4 mg/l, with still higher concentrations (2.4–2.8 mg/l) in colostrum. Rat's milk, according to the work of Cox and Mueller (18), is exceptionally rich in iron and contains about 10 times these concentrations.

There is no evidence for the presence of any special iron compound in milk, but in cow's and human milk all of it, or virtually all of it, exists in an ionizable form which reacts readily with dipyridyl. This is nonhematin, but not necessarily free, ionic iron and it seems highly probable that iron occurs in milk in combination with protein as it does in other body tissues and fluids. Support for this comes from the behavior of the metal in the separation of milk and churning of cream. During the separation of milk, iron distributes itself in direct proportion to the protein and water in each fraction, and during churning it is concentrated in the butter, apparently in the form of an iron protein complex adsorbed on to the fat globule surface (58).

V. Absorption

1. *Mechanism of Absorption*

Since the body's capacity to excrete iron is negligible, some regulating mechanism must exist to prevent excessive amounts of iron from being absorbed over the years and at the same time to permit adequate amounts to enter the tissues. Such regulation is now known to be normally achieved at the port of entry and to reside specifically in the mucosal cells of the gastrointestinal tract. The principle that iron absorption is normally controlled by body requirements was originally put forward by McCance and Widdowson (73) and is supported by an impressive weight of evidence, including studies with radioactive iron. The adjustment of iron absorption to need was, in fact, one of the earliest and most striking conclusions drawn from the application of the radioactive tracer technique with this element. In the healthy normal adult only a small proportion, usually 5–15% of the dietary iron, is assimilated, whereas in an iron deficient subject a much higher percentage of the ingested iron is taken up. In human adults fed radioactive iron, Chodos and Ross (17) found that normal healthy individuals incorporated 1–10% of the administered iron into hemoglobin and 66–90% was recovered in the feces, whereas iron deficient individuals incorporated 40–62% of the same amount of iron into hemoglobin and lost

only 34–67% in the feces Studies by Hahn and others have shown that the efficiency of iron absorption parallels the need for this element in growing children and in the latter half of pregnancy (20) and in dogs rendered anemic by repeated bleeding the amount of iron absorbed was up to 20 times that taken up by normal dogs (44)

Regulation of absorption according to bodily needs does not take place under all circumstances For instance, various conditions including untreated pernicious anemia aplastic anemia hemolytic anemia pyridoxine deficiency hemochromatosis and transfusional siderosis in which serum iron and body iron stores may be either normal high or excessively high have been demonstrated to be associated with increased iron absorption (11, 16 26) In hemochromatosis the increase in absorption must be very considerable to account for the enormous amounts of iron found in the liver of some patients In the investigations of Peterson and Ettinger (94) for instance 20–45% of physiological amounts of radioactive iron given to patients with hemochromatosis was found to be absorbed, compared with 1–4% in normal subjects

The nature of the breakdown of the controlling mechanism in these conditions is not known but it would appear that a condition of anemia can of itself be a factor of some importance whether the anemia is due *to iron deficiency or not. On this assumption the control of iron absorp*tion can be conceived provisionally as being achieved by two mechanisms one is concerned with the absorption of iron into the cells of the mucosa and the other with the passage of iron from the mucosa into the blood stream The first which seems clearly established involves the control of absorption by the amount of iron in the mucosal cell When these cells become physiologically saturated with iron they normally no longer take up iron This is the "mucosal block" hypothesis of Hahn and is experimentally demonstrable Feeding of a dose of iron will result several hours later in a condition which will prevent newly fed iron from being absorbed for some 12 24 hours The presence of iron in the mucosal cells stimulates these cells to form apoferritin to which the iron may rapidly become attached to form ferritin The changes in ferritin content of the intestinal mucosa of the guinea pig following a single oral dose of iron (Fig 3) have revealed how the increase and decrease in ferritin parallel the appearance and disappearance of the mucosal block

It should be noted that the mucosal block theory emphasizes the exclusion of iron from the body to avoid accumulations of harmful amounts It is equally important as Finch *et al* (34) and Josephs (59) have pointed out, to consider this mechanism in terms of the reverse

position Iron is present in foods in such small amounts and is so poorly absorbed that there must not only be a mechanism for limiting its excretion but also to ensure its absorption in sufficient quantities for the requirements of growth and for storage for emergencies

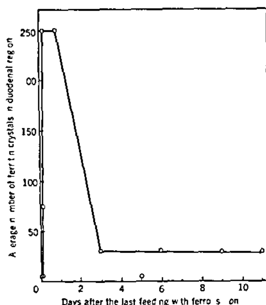
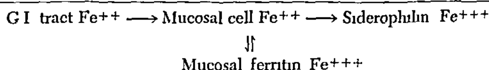


Fig 3 The changes in ferritin content of the duodenal mucosa of the guinea pig after feeding a single dose of iron (Granick 40)

The second mechanism controlling the passage of iron from the mucosa to the blood stream is less convincingly established It is known to be independent of the serum iron or siderophilin levels or of the extent to which the siderophilin is saturated with iron but under certain conditions it is correlated with the oxygen tension of the blood In anemias generally as pointed out in the preceding paragraph iron absorption is increased over that of the normal In normal dogs on the other hand neither anoxia of 46–50 hours duration nor the brief period of anemia following a single large bleeding is associated with an increase in iron absorption (112) Furthermore absorption is increased in growing children adolescents and in the second half of pregnancy without significant hemoglobin reduction The heightened iron absorption of established anemia may be a direct effect of diminished oxygen supply to the mucosal cells or an indirect effect of oxygen tension mediated through a hormonal mechanism influencing the mucosal cells

A completely satisfactory explanation of the nature of the mechanism involved in this aspect of iron absorption and particularly of the significance of such factors as the level of hemopoietic activity and the

avidity of the body stores for iron, clearly must await further experimentation. Nevertheless, the sequence of events in the movement of iron from the intestines to the blood stream can be stated briefly in the following terms: ferrous iron is oxidized to the ferric state following absorption into the mucosal cells, where it attaches itself to the protein apoferritin to form ferritin. At the blood stream end of the mucosal cell the ferric iron is reconverted to the ferrous condition and detached from the ferritin. It passes into the blood stream where it is autoxidized and attached to the protein siderophilin to form serum iron for transport. These events are shown diagrammatically below.



Granick's scheme as modified by Drabkin (25)

2 *Factors Influencing Absorption*

Absorption of iron may take place all along the gastrointestinal tract but in man, the rat and the dog the region of most active absorption is the duodenum, especially in the portion adjacent to the pylorus. No really adequate data exist, however, on the absorption from various levels of the gastrointestinal tract in different species. It appears that to be absorbed, iron must be present as ferrous ions. This is curious in view of the fact that the iron in both mucosal ferritin and plasma siderophilin is in the ferric state. In man studies with radioactive iron have shown ferrous iron to be absorbed more readily than ferric iron (46). In the rat and the dog on the other hand, ferric iron appears to be as well absorbed as ferrous (112, 121). This does not imply that in these species iron is not absorbed as ferrous ions, but rather that reduction of ferric iron to the ferrous state may be achieved more readily in the gastrointestinal tract of these species than in man. It emphasizes the importance also of the existence of gastrointestinal conditions conducive to such reduction.

Production of ferrous ions prior to absorption, from the predominantly ferric iron of the food is presumably a function of reducing agents which are present in the food such as ascorbic acid, reductones and –SH compounds like cysteine but little is known in this regard. Ascorbic acid reduces ferric to ferrous iron *in vitro* and oral administration of ascorbic acid during the feeding of iron salts can increase the efficiency of absorption apparently by reason of its reducing action (95) but

whether it serves this role under ordinary dietary conditions is not definitely known Nor is the role of the HCl of the stomach in iron absorption fully understood Acidity is essential to render food iron soluble and hence ionized and hydrochloric acid is sometimes administered with iron in the treatment of anemia but convincing evidence that it aids the absorption of iron is lacking A large number of individuals who have achlorhydria do not become deficient in iron and patients with pernicious anemia who would be expected to be achlorhydric actually absorb more iron than normal As Granick (40) has pointed out only a very small fraction of the iron ingested in the normal diet is absorbed and this small amount might be absorbed with the help of the organic acids in food which could in part replace the action of HCl When the requirement for iron becomes relatively high however the presence of HCl could be important in making more of the iron of the diet available for absorption

Many other factors materially influence the absorption of iron particularly the chemical form in which it occurs in the food and the nature of the diet in other respects The iron of iron porphyrins such as that of hemoglobin is not available for absorption since iron porphyrins cannot be split or are negligibly split in the gastrointestinal tract to release the iron The iron may exist also in predominantly insoluble or sparingly soluble forms Thus in diets containing abnormally high levels of phosphate the excess phosphate can combine with the iron to form insoluble ferric phosphate which is not available for absorption (13) The presence of relatively large amounts of phytate in the diet has also been stated to have an adverse effect upon iron absorption in man owing to the extremely insoluble ferrous and ferric phytates formed Some doubt has been thrown upon this claim as a result of more recent investigations of longer duration (127)

The possible role of the proteins and their digestion products in influencing iron absorption and its reduction to the ferrous state is suggested by several experimental findings which are little understood In rats the absorption of iron can be greatly increased by the administration of diets in which corn (maize) is the principal source of protein (112) The reduction of iron which takes place when certain foods are mixed with pepsin and HCl *in vitro* (118) has been found to occur equally well in the stomach of normal human beings Breads meat and fruit give reduction of ferric to ferrous iron as high as 50-90% (8)

VI Excretion

Absorbed iron is retained with great tenacity by the body, except for that which may be lost through external blood loss or bleeding into the gastrointestinal tract In fact there is evidence that an appreciable proportion of the very small amount of iron which is excreted is in the form of cellular debris Even the insignificant amount lost in sweat is dependent upon its cell content since dermal excretion of iron is believed to be due primarily to desquamation and not to sweating (3) The limited ability to excrete iron is well illustrated in the treatment of polycythemia with phenylhydrazine and in hemolytic anemia In both these conditions large amounts of iron are liberated in the body by the destruction of red cells yet less than 0 5% of this iron appears in the urine and feces (75) Even when salts of iron are injected intravenously there is only a slight and transitory rise in the level of urinary iron and only a minute proportion of the amount injected is excreted in the urine (74) Loss of injected iron via the feces is also small and slow

Under normal dietary conditions far more iron appears in the feces of healthy individuals than in the urine Earlier values for the urinary iron of normal adult humans were about 1 mg /day or more but more recent work indicates that this is almost certainly too high Barer and Fowler (6) found a group of healthy adults to excrete 0 1–0 3 mg of iron, occasionally more, per day The amount of iron eliminated daily in the feces of normal adults is very variable and depends primarily upon the amounts ingested On ordinary diets the amount usually lies within the range 6–16 mg /day but most of this represents unabsorbed iron True excretory iron in the feces comprises 0 2–0 9 mg /day for normal human subjects by the calculations of Dubach *et al* (27) and averages 0 2 mg /day according to two methods of calculation made by Ingalls and Johnston (57a) An unknown proportion of this excretory iron comes from the bile which appears to be a regular route for iron excretion in normal individuals Actually little is known about iron excretion in the bile in humans but in dogs it has been shown that 0 1–0 2 mg /day is excreted in this way (50) The amount is not greatly affected by raising the iron intake of the dogs either orally or parenterally but it can be increased to 10 times this amount by inducing hemolysis with phenylhydrazine It should be noted that even under these conditions the increased excretion in the bile represents only about 3% of the amount of iron calculated to be released from the hemoglobin of the destroyed red cells and much of this will be reabsorbed from the tract

In women an additional source of iron loss from the body apart from that which takes place to the newborn infant and its adnexa at parturition and in the milk during lactation occurs in the regular menstrual blood losses The periodic blood loss in menstruation is highly variable but averages about 35 ml containing 5 g hemoglobin or 16 mg iron (70) This was formerly regarded as negligible but it represents no less than 0 5 mg iron per day (16 × 13/365) In many women it is appreciably greater

VII Iron Deficiency

1 *Man*

Iron deficiency has been claimed by hospital clinicians to be the most frequently encountered clinically manifest deficiency disease in man It is either of dietary origin or occurs as a result of chronic blood loss The former is most common in infants of either sex the latter occurs usually in adults—rarely in men but fairly often in women

a *The Nature of the Anemic Condition* Surprisingly in view of its importance the pathology of iron deficiency has not been described in detail Darby (19) states that "the iron deficient person is usually rather nervous easily fatigued and listless palpitation on exertion a sore tongue angular stomatitis and dysphagia may be present A hypochromic microcytic anemia is invariably seen Koilonychia may also be seen Any combination of these conditions may be encountered Signs other than the anemia are not usually encountered in children" The oral lesions and nail changes are illustrated in Fig 4 According to Darby (19) these oral lesions occur in one degree or another in only about 10% of iron deficient adults and the koilonychia still less frequently

The anemia of iron deficiency is accompanied by various other changes most of which have been mentioned and discussed earlier and all of which are useful for its diagnosis and discrimination from other forms of anemia The differential diagnosis of the anemias is however more a matter for a text on hematology than on nutrition and will therefore not be discussed further Nevertheless it can be stated that in addition to the hypochromic microcytic condition of the blood iron deficiency anemia is characterized by a normoblastic hyperplasia of the bone marrow and an absence or virtual absence of marrow iron as determined histochemically Also the serum siderophilin levels are low the iron binding protein levels slightly high and the percentage saturation values therefore extremely low The condition is further associated with an increased efficiency of iron absorption judging by the results of a number of studies with radioactive iron

The problem of when an individual or a group should be considered anemic is difficult because of the considerable variation in hemoglobin levels which has been shown to occur in apparently healthy individuals Consistent values 25% or more below normal" are nevertheless, generally considered to indicate an anemic condition On this basis the anemia

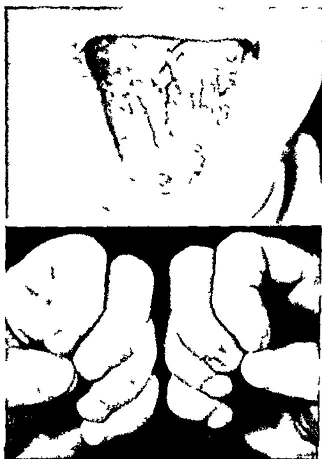


Fig 4 Glossitis angular stomatitis koilonychia in a patient with hypochromic microcytic anemia due to menorrhagia These lesions as well as the anemia responded to therapy with ferrous sulphate alone (Darby 19)

level can be taken to be 12 g per 100 ml in male adults and 10 g per 100 ml in female adults Such a deficiency, especially when it develops slowly is usually well tolerated Not until the hemoglobin is reduced to 50–60% of normal (8–9 g in males and 7–8 g in females) is functional embarrassment usual although such figures are arbitrary and elastic and cannot be applied to all individuals in all circumstances

b *Iron Deficiency Anemia in Infants* In the absence of iron supplementation the hypochromic anemia of infancy occurs most frequently

in children from 6 to 24 months of age There is no doubt that this *is an iron deficiency anemia in the sense that it responds rapidly to* iron therapy but its etiology is by no means as simple and straightforward as was formerly supposed According to the classical theory of Bunge (14) which has dominated thinking on this subject for half a century mammals are born with a congenital store of iron in their livers normally *sufficient to compensate them for the low intake of iron* from the maternal milk during suckling It is now known that milk in spite of its low iron content is a far from negligible source of iron and that a diet of milk alone up to 12 months of age could not account for more than a very moderate degree of iron depletion in the human body It can be shown further that the congenital liver stores of most mammals (the rabbit is an exception) are insufficient to make more than a small contribution to their iron needs (76) For example the liver of the newborn human infant usually contains no more than 30–40 mg of nonhemin iron which is only about one third to one half of the amount of iron ingested from the milk during the first six months (77)

The major source or "store of iron in the human infant is normally contained in the considerable plethora of hemoglobin in the blood at birth If the blood of the newborn contains 20–22 g hemoglobin per 100 ml which is usual provided that the tying of the cord is delayed until it has stopped pulsating (83) and the blood volume is taken as 300 ml it can be calculated that some 200–220 mg of iron is present in the blood alone or 6 to 7 times the amount stored in the liver At the end of the first six months the average human baby has increased in weight from 3 5 to 7 kg the hemoglobin content of its blood has fallen to about 12 g per 100 ml and its blood volume per unit of body weight has decreased slightly from the newborn level (83) There is therefore about 280 mg iron in the form of hemoglobin in the blood of the six months infant of which some 200 mg was present at birth *Since a high proportion of this would be released from hemoglobin* breakdown and retained for the needs of the body it must make a very substantial contribution indeed to the iron required by the infant for growth and hemoglobin production In fact the total stores of iron in the newborn baby s blood and liver together with the iron retained from the milk are such as to make it difficult to visualize iron deficiency arising at all Clearly the whole problem requires reappraisal in these terms This has recently been done very ably by Josephs (59) who emphasizes that the hypochromic anemia of infancy is not normally associated with depletion of iron stores the baby he argues may be "starving for iron in the midst of plenty "

No really adequate hypothesis or hypotheses to account for the iron deficiency anemia of infancy have yet been put forward Josephs examined the position from the point of view of the influence of pregnancy, diet, growth, infections extra uterine adjustments and congenital factors but arrived at no really adequate conclusions There is some evidence that the babies of anemic mothers have slightly lower levels of hemoglobin at birth and smaller concentrations of iron in their livers than those from healthy mothers (115) The liver iron stores of premature infants are also smaller than those of full term babies (77) because most of this iron is deposited during the last third of pregnancy These observations have not been well correlated with the later onset of anemia except perhaps with premature babies and in these cases it seems likely that the main factor is their more rapid growth rate compared with that of normal infants The possibility of copper deficiency has been raised but although some successes from copper therapy with iron have been reported a great majority of cases respond to iron alone More speculative but of great physiological interest, is the question raised by Josephs of competition for iron between tissues and hemoglobin during growth Iron is needed in the tissues as part of the building material especially in the muscles but may also be diverted to the tissues as a physiological factor in accelerating their growth This demand for iron by the tissues of the growing infant could take precedence over the demands for the increasing volume of blood and compete successfully where the supplies or the intakes of iron are limited Suggestive evidence of the effectiveness with which the tissues compete for iron in the infant is provided by the absence of epithelial changes associated with the anemia at this age whereas such changes occur in the hypochromic anemia of adults (Fig 4) in consequence it has been proposed of tissue iron deficiency (102)

Speculative suggestions of this sort must remain in the realm of ideas until further investigations either support or invalidate them There is however no question of the effectiveness of iron therapy in the hypochromic anemia of infancy and of the necessity for protection against iron deficiency by the supplementation of a milk diet with iron rich foods at least from the age of about 12 months and considerably earlier with premature babies

c *Iron Deficiency in Adults* It was stated earlier that iron deficiency in adults occurs principally as a result of chronic blood loss but rarely in males Iron deficiency does not often occur as a result of a single massive hemorrhage but may result in either males or females from chronic blood loss due to pathological conditions such as bleeding

peptic ulcers hemorrhoids hookworm infestation or secondary to malignant changes in the gastrointestinal tract, nasopharynx or urinogenital tract The female is subject however, to three important sources of loss to which the male is not subject—menstruation gestation and lactation There is no clear evidence of iron deficiency anemia resulting from lactation although the amount of iron excreted in the milk may amount to 0 5 mg /day or more for some months but it can occur in many women as a result of either menstruation or pregnancy

Normal females between the ages of 13 and 47 years escape menstruation only by occasional pregnancy From the standpoint of avoiding loss of body iron this is unprofitable because some 350–450 mg of iron is lost in the fetus and its adnexa compared with a total of about 200 mg yearly in the menstrual flow Compensation for the increased iron demands of pregnancy is apparently achieved to some extent by increased absorptive efficiency but there is no proof that this is sufficiently effective to prevent iron deficiency in late pregnancy in many women on ordinary unsupplemented diets According to Dieckmann and his colleagues (22) nearly 40% of women have a low grade anemia during pregnancy and would benefit from iron therapy Nor is the loss of iron in the menses negligible from this point of view An extra retention of 0 6 mg iron per day requires an appreciable share of the dietary iron but from the extensive data of Frenchman and Johnson (38) it is apparent that this applies to only about 50% of women In about 15% of the women studied definitely larger menstrual losses were observed which imposed a replacement need of over 1 mg iron daily This is by no means easy to supply from ordinary unsupplemented diets To avoid iron deficiency it may therefore be advisable to supplement with iron the diets of 10–15% of "normal women

d *Therapy* The treatment necessary to prevent or overcome iron deficiency anemia varies with its cause In infants it can best be prevented by providing the mother during late pregnancy with a diet well supplied with iron and other nutrients and by the introduction of a variety of foodstuffs into the baby's diet during the second half of the first year of its life Once iron deficiency has developed therapy *with a suitable preparation of a ferrous salt is necessary in addition* to the dietary improvements Treatment of established iron deficiency by ordinary dietary means alone is a long and doubtful process at any age

For the prevention of iron deficiency in the adult it is necessary to detect and where possible correct the sources of chronic blood loss and to administer orally a readily absorbed preparation of ferrous iron

Large doses are usually necessary for the best clinical results but as the efficiency of absorption decreases with increasing dosage there is clearly a limit beyond which the iron is merely wasted A daily dosage of not more than 350 mg of ferrous sulfate plus the simultaneous administration of ascorbic acid to aid absorption is commonly used For quicker results in view of the low absorption of orally administered iron intravenous injection of suitable preparations such as saccharated iron oxide is being increasingly practiced Such treatment must be carried out with circumspection because of the danger of toxic effects and the development of hemosiderosis Good results especially in the anemia of pregnancy with very few toxic reactions, have been obtained by these means with a total dosage of 25–40 mg of elemental iron for each 1% deficit of hemoglobin and a limit of 100 mg for a single dose (55, 103)

2 *Farm Animals*

a *Pigs* Pigs are the only farm animals in which iron deficiency is known to present a practical problem Even in this species it is confined to the first part of the suckling period and to animals reared under very restricted conditions Braasch, the pioneer worker in this field reported an extraordinarily high death rate among suckling pigs in Schleswig Holstein due to anemia and stated that the disease became especially common after the so called modern hog house came into use (12) McGowan and Crichton (80) in the United Kingdom and Doyle and co workers (24) in the United States subsequently showed that piglet anemia was exceedingly prevalent in pig breeding establishments in those countries where the sows were housed in pens with concrete floors without access to soil or pasture More recent investigations (49 126) have established the disease as an uncomplicated iron deficiency with a number of interesting features in relation to iron metabolism It can be overcome by direct administration of iron salts to the piglets or by farrowing under less restricted conditions so that additional iron can be obtained by the piglets from soil or pasture

Piglet anemia is often referred to as thumps because of the labored, spasmodic breathing which characterizes the condition It generally develops within 2 to 4 weeks of birth by which time the hemoglobin level of the blood has fallen from a normal 8–9 g per 100 ml at birth to as low as 3–4 g per 100 ml A high proportion of affected litters die but surviving members usually begin a slow spontaneous recovery at about 6 to 7 weeks of age by which time they have normally started to consume significant quantities of the sows food and to undertake what

liver iron stores (Table 3) The amount of iron in the pig's liver at birth is therefore even more inadequate for the rest of the requirements of the body during the suckling period than is the liver iron for this purpose in other species And thirdly, the pig in marked contrast to other mammals for which data are available is not born with a plethora of hemoglobin in its blood In fact the hemoglobin concentration of the blood of the healthy pig is very similar at birth at weaning and at maturity The absence of this source of endogenous iron which was shown to be so important to the human infant, together with the very rapid growth rate are considered to be highly significant factors contributing to the onset of suckling anemia in the pig They show clearly why this condition can arise so much more readily in this species than in the ovine bovine or human suckling

b *Poultry* Iron is obviously necessary for hemoglobin synthesis in birds just as it is in mammals but there is no conclusive evidence of the natural occurrence of iron deficiency anemia in either growing chicks or laying hens Indeed the normal diets of these animals appear to supply abundant iron for their requirements There is no doubt that under certain conditions, anemia is a cause of embryonic mortality during incubation (108) but either direct summer sunshine or access to a grass range alleviates the condition and its incidence has not been correlated with the iron (or copper) content of the eggs (28) Nor is it possible to raise the iron content of the egg by feeding extra iron to the hen In the laying hen the demand for iron for egg formation is large since the average egg contains about 1 mg of this element and there is some evidence that anemia accompanies heavy egg production However this anemia does not appear to be associated with lack of iron Schultze and co workers (101) found a practical laying ration to supply about 14 mg of iron per hen per day which could support heavy egg production without reducing the hemoglobin level of the blood Moreover the feeding of additional iron did not raise either the hemoglobin level or egg production In spite therefore of the very rapid growth rate of chicks the remarkable productive performance of the modern laying hen in relation to her size, and the high iron content of eggs commonly fed rations supply more than sufficient iron to prevent the occurrence of iron deficiency

c *Sheep, Cattle and Horses* Areas exist in different parts of the world where the soils are so low in available iron that the herbage has been reported to carry insufficient iron for the requirements of grazing stock resulting in emaciation and anemia The iron deficiency theory used to explain these disorders of sheep and cattle appeared to be

supported by the finding that supplementation of the diet with large amounts of many iron compounds was completely effective in preventing or curing the condition Filmer and Underwood in Western Australia showed this theory to be untenable since they were able to cure the condition by "iron free" extracts of a potent iron compound (33) This and other relevant findings are discussed later and it will suffice at this stage to state that deficiencies of cobalt or copper or both in the soils and herbage of the so called iron deficiency areas were later shown to be the real cause of the anemia and other symptoms of disease The efficacy of the iron compounds used resides in the cobalt which they contain All crude iron compounds so far examined are contaminated with variable amounts of cobalt (120)

Iron deficiency under naturally occurring conditions has never been demonstrated in sheep cattle or horses and seems impossible of occurrence in view of the very liberal amounts of iron carried by most feeds relative to any conceivable requirements The iron requirements of these species have never been determined but pasture herbage very rarely contains less than 80 p p m of iron on the dry basis (122) and 150 to 250 p p m are more usual concentrations (107) Even the cereal grains notorious for their low status in many minerals contain relatively liberal amounts of iron which fall mostly within the range 30–60 p p m (7) On the basis of these figures a 50 kg grazing sheep consuming a 1000 g of dry matter daily would ingest from the herbage alone that is excluding the considerable possibilities of additional iron from soil contamination a minimum of 80 mg iron per day This is some 5 to 10 times the amount of iron ingested daily by adult men and women on typical modern dietaries!

Even during the suckling period lambs calves and foals are unlikely to develop iron deficiency because of the conditions under which they are normally reared In common with other mammals they have relatively high liver iron stores and blood hemoglobin levels at birth (78 81) but these at least in the lamb (116) are quite inadequate to prevent severe anemia developing within 6–8 weeks if they are restricted to a milk diet under confined conditions The young of these species normally begin to consume appreciable quantities of feeds which are very much richer in iron than the milk of their mothers at relatively early ages usually within about a month of birth This effectively prevents the development of iron deficiency

VIII Requirements for Iron

1 *Man*

Single figures for the iron requirements of humans of different ages must always be arbitrary and approximate because of variations in requirements from person to person and in iron availability from foodstuff to foodstuff and from diet to diet. Numerous iron balance studies which have yielded much valuable data have been carried out over many years but these are subject to technical difficulties which limit their accuracy even with the elegant radioactive iron techniques of recent years. Moreover, reliable balance data for successive years of life are not available. Iron requirements can also be deduced from the physiological demand multiplied by a factor to adjust for the percentage absorption of alimentary iron. Such calculations have been made by Drabkin (25) *and agree fairly well with the suggested daily dietary* allowances of 1 mg/kg for infants, 0.6 mg/kg for children, 10 mg for adult males, 12 mg for adult females, and 15–20 mg for pregnant females (92). Calculations from physiological demand are particularly effective in revealing changes consequent upon rapid growth as in males at puberty or upon pregnancy and menstruation in females but they make no allowance for differences in percentage absorption of iron between individuals and between diets or for increased absorptive efficiency in particular conditions such as iron deficiency or pregnancy.

The physiological demand for iron is largely determined by the requirement for hemoglobin turnover, for hemoglobin growth (accretion during growth), and in the female, between puberty and the menopause for the replacement of iron lost in menstruation, gestation, and lactation. Hemoglobin turnover is an overwhelmingly larger metabolic process than either hemoglobin accretion in growth or its loss in menstruation, but most of the iron (97–98%) from the catabolized hemoglobin *is conserved so that its replacement makes very little demand on dietary* iron. In fact, as was indicated earlier in relation to excretion, the healthy male adult excretes only about 0.9 mg iron daily, of which about 80% or 0.7 mg/day represents hemoglobin iron. Hemoglobin growth in childhood and adolescence and losses of blood and iron in menstruation and gestation, on the other hand, make relatively large demands on exogenous iron although the amounts of hemoglobin involved are so much smaller than the amounts being catabolized in hemoglobin turnover. Moreover, these physiological demands on exogenous (dietary) iron are additional to the small requirements consequent upon the slightly imperfect conservation from hemoglobin turnover which exist in every individual.

The best estimates of iron requirements based on calculations of the iron needed to fulfil these physiological demands are still those of Heath and Patek (53) made in 1937. More recent estimates for full term babies up to 24 months of age and for premature babies up to 12 months of age are given by Josephs (59). Heath and Patek's figures are summarized in Table 7.

Heath and Patek's data are far from perfect but they indicate that there are three periods when the requirements for iron are greatest both

TABLE 7

IRON REQUIREMENTS FOR GROWTH, MENSTRUATION AND PREGNANCY[a]

	Total annual requirement (mg Fe)	
Year of life	Males	Females
1	195	182
2	112	112
3	80	92
4	92	80
5	98	87
6	79	106
7	80	78
8	70	67
9	72	108
10	152	120
11	130	163
12	137	164
13	189	192
14	198	145
15	314	468
16	313	424
17	353	498
18	183	435
19	149	452
20	81	365
21	71	358
22	–	298
23	–	298
24	–	298
25	–	374[b]
26	–	298
Total Requirement (g Fe)		
Birth to 21 years	3.15	4.59
Birth to 47 years		12.22

[a] From Heath and Patek (53)

[b] Calculated for pregnancy

relatively and absolutely They are during the first two years of life during the rapid growth of adolescence and throughout the childbearing period in women The very great increase in requirement in males at age 15–16 years due to the extraordinary rapid hemoglobin accretion at this time (see Fig 1) the much higher requirement of females than males from the menarche at age 14 onwards and the exceedingly low requirement after growth ceases in the male are particularly evident

It should be emphasized that these are not estimates of *dietary* requirements those have been given in the first paragraph of this section

2 *Farm Animals*

Practically nothing is known of the iron requirements of farm stock The abundance of iron in all ordinary farm feeds and the absence of an iron deficiency problem in these species other than in suckling pigs no doubt explains the marked lack of interest of nutrition workers in this question No conventional iron balance studies with sheep cattle or horses appear to have been carried out, nor so far as is known have the great possibilities of radioactive iron in metabolic investigations with these species been exploited Rough estimates of the physiological demands for iron for hemoglobin turnover and for accretions of hemoglobin and other chromoproteins in growth could be made in much the same way as has been done for humans but the conversion of such estimates into dietary requirements is impossible until data have been obtained on their absorptive efficiency Assumptions that iron absorption is regulated in the same way in the ruminant as in man or the dog are completely unwarranted in view of the very great differences known to exist in other aspects of the digestive and absorptive processes in these species The whole problem of iron metabolism in herbivorous farm animals especially ruminants appears to present an interesting and rewarding field for research

IX Sources of Iron in Foods and Dietaries

There have been many analyses of the iron content of foods, from which tables of average composition of representative foods grown or processed under a wide range of environmental conditions have been compiled Such comprehensive compilations have been made in recent times with acceptable methods of analysis for U S (123) English (85) Australian (91) and Indian (52) foods The results are characterized by great variability from sample to sample of the same food material the variability being related to variety soil type climate manurial treatment and many other factors Nevertheless consistent

differences exist in the iron content of the edible portions of various foods which have considerable nutritional importance Relatively rich sources of total iron are the organ meats (liver kidney and heart) egg yolk dried legumes, bran cane molasses or black treacle cocoa shellfish dried yeast and parsley Poor sources of iron include milk and milk products fats and oils white bread and flour (unenriched) polished rice sago white sugar potatoes and most fresh fruit Foods of intermediate iron content are the muscle meats fish and poultry nuts green and green leafy vegetables wholemeal bread flour and cereals and enriched bread and flour

Vegetables and fruits which are classed above as either poor or medium sources of total iron are grouped by Stiebeling (113) as follows

Poor (0–4 p p m edible portion)	most fruits and fruit juices
Fair (4–8 p p m edible portion)	some fruits seed pods blanched leaves and stalks roots and herbs
Good (8–16 p p m edible portion)	potatoes thick green stalks and leaves
Excellent (over 16 p p m edible portion)	mostly leguminous plants and leaves

These levels of iron may be greatly reduced by boiling in water Losses of 20% or more have been reported by such treatment (107)

The influence of the degree of extraction in the milling of wheaten flour upon the iron content of the product and the value of iron enrichment of low extraction flour can be gaged from the following representative figures

Patent flour (60% extraction)	7 p p m iron
White flour (70% extraction)	9 p p m iron
80% flour	16 p p m iron
85% flour	21 p p m iron
Wholemeal flour	30 p p m iron
Patent or white flour (enriched to "standard")	29 p p m iron

Iron exists in foods in a variety of chemical forms some of which are completely available for absorption and some of which can be absorbed with difficulty or not at all This has led to numerous studies of "available" iron by the use of the dipyridyl reagent of Hill (54) which does not react with hematin iron Such determinations were based on comparatively early evidence showing that neither hemoglobin nor hematin iron is absorbed presumably because it is not detached in the gastro

intestinal tract from the porphyrin complex, of which it forms a part Even this assumption is apparently not fully justified in the light of the finding of Black and Powell (9) that 10–20% of hemoglobin iron was absorbed when a liter of citrated blood was given by duodenal tube The absorption was considered to result from bacterial decomposition of the blood in the intestine However determination of the available (nonhematin) iron of foods enjoyed a considerable vogue for a number of years and was supported by the good correlation shown to exist between the available iron in various foods and the value of these foods for hemoglobin synthesis in rats (105) No such correlation has yet been demonstrated in man and even in the rat some curious and un explained discrepancies have been found (109) It is questionable, there fore, whether available iron determinations of human foods are of much real value, and they are now much less frequently used In this con nection it is as well to point out that hematin compounds are not the only organic compounds of iron which do not react with dipyridyl on reduction In many foods and particularly in fruits and vegetables hematin compounds may account for only a small fraction of such non reacting iron as Hill (54) pointed out in his original contribution Nothing is known of the availability for absorption of these compounds in man Finally it may be stated that the chemical nature of iron compounds in foods is probably much less important for their absorp tion than other less easily defined factors the most important of which have been discussed when considering iron absorption

The wide range in iron content of different foodstuffs indicates clearly that the over all iron intake from different diets will vary greatly accord ing to the proportion of iron rich and iron poor foods which they contain The cheapest and most readily available and consumed foods in terms of *calories, are the carbohydrate rich materials notably* the milled cereals white sugar and potatoes These are all low in iron and they invariably constitute a high proportion of low cost diets where the choice of foods is dictated largely by the need to satisfy immediate hunger rather than by the more subtle needs of the body Improvement of the quality of such diets by the inclusion of more dairy products is nutri tionally highly desirable but it does not increase and may even decrease their over all iron content Improvement in iron content requires in creased proportions of meat especially organ meats eggs green leafy vegetables leguminous seeds and whole cereal or high extraction cereal products All of these foods are normally much richer in iron than those just mentioned as being consumed in large amounts in low cost diets

The importance of this in human nutrition is revealed by a number of

studies of actual dietaries in different parts of the world. A diet typical of that consumed by millions in India has recently been shown to supply only 9 mg. of iron daily (52), whereas an improved diet composed of local materials but containing a much lower proportion of milled rice and increased amounts of pulses and green leafy vegetables will supply no less than 60 mg. iron daily (52). An average of 11 mg. iron daily was shown some years ago to be ingested in the dietaries of the poorest classes in Aberdeen (21). This was quite inadequate for women since anemia was prevalent among nearly half the women in the survey (and this anemia responded remarkably to iron therapy) but it was presumably satisfactory for most men and children because anemia was found to be rare in them. Average American dietaries were shown by Sherman (104) to supply from 14 to 20 mg. iron per man value daily, and Australian dietaries where the proportion of meat is unusually high to provide 20–22 mg. iron per adult male daily (90). These appear to be ample for all ordinary requirements including those of women during the childbearing period but average figures of this sort can obscure important differences between individuals, families and groups. Thus Stiebeling and co-workers (114) in their extensive studies of family food consumption and dietary levels in the United States demonstrated significant differences in iron intake in groups of families grouped according to their level of expenditure on food. At the three levels of expenditure included the iron intake per nutrition unit averaged 12, 14 and 16 mg per day with increasing expenditure on food. Similarly even in Australia where economic conditions are generally very good and iron intakes relatively high it has been found that the daily intake per nutrition unit falls with increasing size of family from an average of 15 to an average of 11 mg. iron per day (90).

An inescapable conclusion from the above findings is that almost any *diet likely to be consumed by man will supply ample iron for adult* males but that many diets, especially where the expenditure available for food limits the choice to low cost materials high in energy, provide insufficient iron for women and in certain circumstances, to be discussed in the following paragraph, for growing children because of their much higher requirements. Improvement of such diets in relation to iron can be achieved at little extra cost either through a more judicious choice of foods or by iron medication.

In the preceding paragraphs emphasis has rightly been placed on the general nutritional quality of the diet as the principal determinant of its iron content. There are circumstances however in which the source as well as the choice of foods can greatly influence iron intakes

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ataxia was a manifestation of copper deficiency which could be prevented by the administration of copper to the ewes during pregnancy These pioneer discoveries were followed by the demonstration of extensive copper deficiency areas in many parts of the world—areas in which (a) sheep and cattle failed to thrive unless supplied with extra copper either directly or indirectly through the pastures (b) the liver and blood of affected animals were greatly below normal in copper content and (c) the herbage and in most cases the soils contained subnormal levels of copper

While these investigations were proceeding it was demonstrated that copper is an essential dietary factor for normal pigmentation of hair fur and wool in several species and for the keratinization process in the growth of wool Such findings and particularly the great economic significance of naturally occurring copper deficiency conditions gave a tremendous stimulus to studies of copper in nutrition—studies which have revealed how widely this element is concerned in bodily processes and how closely its absorption utilization and excretion are linked with other dietary components notably iron and molybdenum These investigations were greatly facilitated by the contemporary development of reliable methods of estimation of the very small amounts of copper involved Many of the earlier results are open to grave suspicion because of faulty analytical methods and the failure to take sufficient precautions to avoid contamination with copper

II Copper in Animal Tissues and Secretions

1 *The Copper Content of the Whole Body*

The healthy adult human body contains about 100–150 mg of copper or 1 5–2 p p m (29) This is very much lower than the total amount of iron and only about one tenth of the amount of nonhemoglobin iron normally present Very similar concentrations of copper occur in the whole bodies of the adults of most other species No data for the whole bodies of normal adult sheep or cows are available but it is probable that they contain appreciably higher concentrations because of the exceptionally high levels of liver copper which usually occur in these species Newborn and very young animals invariably contain 2 to 3 times more copper per unit of body weight than adults of the same species although as with iron there is more species variation at this early stage of development than at maturity (Table 8)

The whole body of the newborn calf is apparently much richer in copper than that of any of the species given in Table 8 Calculations from the data of Rusoff (113) give a concentration of about 14 p p m

detected this element in snails in 1847 and showed that it existed in combination with the blood proteins Fredericq (59), in 1878 established the fact that the copper containing pigment (hemocyanin) of the blood of the octopus behaved as a respiratory pigment Hemocyanins from different marine species are now known to vary in composition and in copper content but they all unite with oxygen in a definite ratio to copper One atom of oxygen is taken up for each atom of copper in the hemocyanin molecule

A different type of copper compound which aroused early biological interest is the colored pigment, turacin, found in the feathers of the South African bird, turaco It was first described by Church (30) in 1869 who later found it to be analogous to hematin and to contain no less than 7% of copper It has since been shown to belong to a derivative of the porphyrin pigments which are normally found in plant and animals (111a) but the turaco is apparently the only species in which copper occurs combined with porphyrin No reason for its occurrence in these birds is yet known

Following the demonstration of the essential role of copper in hematopoiesis, although largely independent of it, there was a great upsurge of interest in the biological function of copper A number of copper protein compounds were isolated from both plant and animal sources several of which were shown to be enzymes with an oxidase function [tyrosinase (polyphenoloxidase), laccase, and ascorbic acid oxidase] (75 77, 78) More recently butyryl CoA dehydrogenase has been identified as a cuproflavo protein in which copper occurs as part of the prosthetic group of this enzyme (91) The activities of these enzymes are dependent upon the copper they contain but little is yet known about how the copper is bound or how it functions within the enzyme Nor is it yet clear what quantitative significance can be attached to these copper containing enzymes in the oxidative processes of the intact cell

Of equal importance were the investigations, which began in the early 1930s of certain naturally occurring diseases of grazing sheep and cattle which were shown to be caused by a deficiency of copper, or of copper and cobalt or to respond to copper therapy The first published report suggesting that deficiency of copper occurs naturally in livestock was that of Neal and associates in 1931 as a result of their studies of "salt sick of cattle in Florida (108) In 1933 Sjollema in Holland showed copper deficiency to be a causal factor in a wasting disease of sheep and cattle characterized by diarrhea loss of appetite and anemia and called lechsucht (128) In 1937 Bennetts and Chapman (14) in Australia showed that a demyelinating disease of lambs named enzootic

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The high levels of the newborn are largely, but not entirely, a reflection of the high liver copper storage which characterizes this growth stage In most species such levels are maintained during the suckling period and are followed by a steady fall during growth from weaning onwards until adult levels are reached

TABLE 8

CONCENTRATIONS[a] OF COPPER IN THE WHOLE BODIES OF DIFFERENT SPECIES

	Human	Pig	Cat	Rabbit	Guinea pig	Rat	Mouse
Newborn[b]	4 7	3 2	2 9	4 0	6 9	4 3	6 7
Adult[c]	1 7	2 5	1 5	1 5	—	2 0	—

[a] Measured in p p m of fat free body tissue
[b] E M Widdowson (140a)
[c] C M Spray and E M Widdowson (131a)

Data on the influence of sex on the copper content of the body are extremely limited but sex differences if they exist, must be very small

The distribution of the total body copper among the tissues can only be given in very general terms because of the paucity of reliable data and the very wide differences in the results of different investigators Chou and Adolph (29) estimate that, of the 100–150 mg found in the adult human body, 65 mg is found in the muscle mass, 23 mg in the bones and 18 mg in the liver In the adult rat fed a normal stock diet 21% of the total body copper has been found in the muscles 23% in the bones 13% in the liver, and 36% in the skin (87) In one newborn calf 18% was found in the muscles, 54% in the bones, 12% in the liver, and 2 3% in the skin, whereas in another newborn calf examined by the same methods by the same workers (113), the distribution of total body copper was as follows 26% in the muscles, 7% in the bones 12% in the liver, and 17% in the skin In two adult sheep which had exceptionally high liver copper stores, Dick (45) found that the distribution was 72–79% in the liver, 8–12% in the muscles 9% in the skin and wool and about 2% in the skeleton

These results, in spite of their great variability emphasize the large total *amounts* of copper which can occur in the bones and muscles and the skin of haired animals as well as in the liver

2 *Copper in Various Tissues and Organs*

Data are available for the copper content of the principal tissues and organs of the rat rabbit cat dog pig guinea pig sheep, cow horse domestic fowl and man Varying concentrations have been found in every tissue and organ examined and there is good reason to believe that

copper occurs in all body cells The individual variability is large—too large in fact to allow definite values to be assigned to particular organs Nevertheless in all species certain tissues and organs consistently carry higher concentrations of copper than others and in general tissues or organs which are high or low in one species are similarly high or low in others (23 36 88 93 95) as shown in Table 9 The endocrine glands (pituitary thyroid and thymus) are examples of organs with very low concentrations of copper whereas the liver heart kidneys hair and brain roughly in that order are usually among the highest in this element The spleen and the lungs represent organs of intermediate copper content The muscles and bones (particularly ribs) are tissues for which some surprisingly high values have occasionally been reported but which *normally carry lower concentrations of copper than the spleen* the lungs or the skin The high total amounts of copper present in the muscles and the bones result not from high concentrations but from the large mass of these tissues which exist in the body

From the limited evidence available it appears that neither a deficiency nor an excess of dietary copper influences greatly the copper content of certain of these tissues and organs notably the endocrine glands the muscles the brain the heart and the skin The copper content of the liver kidney, spleen and lungs by contrast can be greatly increased by high copper intakes and that of the liver kidney spleen hair and blood greatly reduced under conditions of copper deficiency Thus Lindow *et al* (87) found that the addition of 5 mg of copper per day to a normal stock ration raised the copper concentration of the livers of adult rats from 11 to 213 p p m on the dry basis the kidneys from 12 to 17 p p m and the spleen from 3 to 17 p p m whereas in the same animals there was little change in the levels of copper in the heart brain muscles and skin

In certain disease conditions in man namely Wilson Uzman s disease (hepatolenticular degeneration) and hemochromatosis exceedingly high concentrations of copper far beyond those regarded as normal occur in the liver brain and other tissues In Wilson Uzman s disease these appear to be related to exceptionally high copper absorption rather than to high intakes

3 *The Copper Content of the Liver*

The liver has received far more attention than any other organ or tissue because following the early lead given by McHargue it soon became evident that it acts as the main storage organ of the body for copper and can provide a reasonably reliable index of the copper status

TABLE 9

COPPER CONTENT[a] OF ORGANS OF DIFFERENT SPECIES (CUNNINGHAM 36)

Species	Description	Liver	Heart	Lungs	Spleen	Kidney	Pancreas	Brain	Flesh	Skin	Hair
Human	Adult[b]	24.9	—	—	5.2	17.5	4.3	17.5	—	—	
Bovine	Adult[c]	77.0	15.6	5.3	2.9	19.7	3.8	—	—	—	
Bovine	Newborn	470.0	14.6	4.9	4.8	15.7	8.5	—	1.8	—	
Bovine	Fetus	262.8	10.4	3.6	5.4	8.5	—	—	2.9	2.1	
Sheep	Adult	236.6	17.9	9.6	5.0	17.8	7.7	—	—		
Horse	Adult[c]	14.8	17.6	6.8	3.2	28.9	—	—	—		
Pig	Adult[c]	41.3	14.9	5.3	6.0	21.1	—	—	—		
Pig	Few days old	232.8	12.8	3.4	6.8	14.7	—	—	—		
Dog	9 days old	98.2	17.4	6.2	—	14.2	—	8.5	—	9.9	22.7
Cat	Adult[c]	25.3	14.4	3.8	5.2	10.1	—	14.6	2.3	4.2	11.9
Guinea pig	Adult	17.0	21.2	9.5	—	19.9	—	—	—		—
Rabbit	Adult	9.2	22.3	8.1	—	13.7	—	—	—		—
Rat	About 90 *g* weight[c]	10.0	27.8	9.5	8.1	22.6	—	10.2	3.8	7.3	14.8
Badger	Adult[c]	21.7	12.8	5.6	3.0	9.4	—	10.8	—	3.2	—
Domestic fowl	Adult[c]	12.4	14.9	2.4	—	11.7	—	—	—	—	4.9[d]
Average		103.6	16.8	6.0	5.0	16.4	—	—	—	—	—
Range		9.2–470	10.4–27.8	2.4–9.5	2.9–8.1	8.5–28.9	—	—	—	—	—

[a] Measured in p.p.m. on the dry basis

[b] Average of 3 series of analyses

[c] Average of 2 series of analyses

[d] Feathers

of the animal Liver copper concentrations have been determined for an exceptionally wide range of animal species including mammals, birds fish reptiles and amphibia (7) In addition many workers have studied within a much more restricted range of species the factors which influence liver copper levels It is apparent from these studies that the concentration of copper in the liver is determined by four main factors which are considered below, namely the species the age of the animal the nature of its diet and the occurrence of certain disease conditions (Table 10) In the Australian salmon (*Arripis trutta*) Beck (7) has shown that there is a highly significant sex difference but there is no evidence of a significant influence of sex on liver copper in other species

a *Species Differences* In spite of appreciable individual variation which is common to all species a number of interesting species differences exist in the normal range of copper in the liver The livers of normal adults of most species have been shown by Beck (7) usually to contain between 10 and 50 p p m of copper on the dry basis with a high proportion of individual values lying within the narrower range of 15–30 p p m These levels apply to species as unrelated and as diverse in their environment and their nutritional habits as man rats, rabbits cats dogs foxes pigs kangaroos whales crocodiles snakes domestic fowls turkeys emus sharks and herring In contrast to this remarkable uniformity, much higher liver copper levels are exhibited by a few species Examples of such species are the sheep cow duck frog and several species of fish for which the large normal range of 100–400 p p m may be given (Table 10)

No satisfactory explanation of these differences can be given on the basis of present knowledge Differences in copper intake alone can hardly suffice since domestic fowls turkeys and ducks consume very similar diets and yet the two former species normally carry very much lower levels of copper in their livers than do ducks Moreover the whale which lives on marine organisms high in copper is included in the group with low liver copper storage It seems likely that physiological differences in the efficiency of copper absorption excretion and storage will be found to lie at the root of these interesting species variations

b *The Influence of Age* In all species for which adequate data are available with the exception of the sheep and the cow liver copper concentrations are much higher in the normal healthy newborn than they are in the normal healthy adult (Table 10) In the human species average values for the newborn are 5 to 10 times those of the adult During intra uterine life considerable storage of copper occurs in the liver but the extent of this storage and the time at which maximum

TABLE 10

THE INFLUENCE OF SPECIES AGE AND COPPER INTAKE ON THE CONCENTRATION OF COPPER IN THE LIVER

Species	Age and treatment	Number of animals	Copper concentration (p p m)[a]	Reference
Man	Newborn (0–7 weeks) normal		230	Bruckmann and Zondek (23)
Man	Adult normal diet		35	Bruckmann and Zondek (23)
Rat	Newborn normal diet	30	58 ± 4 0	Lorenzen and Smith (88)
Rat	Mature normal diet	10	9 ± 0 4	Lorenzen and Smith (88)
Rat	Mature stock diet plus copper	—	213	Lindow *et al* (87)
Rat	Young copper deficient diet	—	3	Schultze *et al* (122)
Rabbit	Newborn normal	30	37 ± 6 7	Lorenzen and Smith (88)
Rabbit	Mature normal diet	10	23 ± 3 6	Lorenzen and Smith (88)
Rabbit	Mature normal diet	7	15 0 ± 0 63(14–19)	Beck (7)
Guinea pig	Newborn normal	30	67 ± 5 6	Lorenzen and Smith (88)
Guinea pig	Mature normal diet	10	23 ± 3 5	Lorenzen and Smith (88)
Guinea pig	Mature males normal diet	44	77 3 ± 5 6(29–205)	Beck (7)
Pig	Newborn normal		233	Cunningham (36)
Pig	Mature normal	12	18 9(12–48)	Cunningham (36)
Pig	Mature normal diet	14	18 9 ± 0 87(15–25)	Beck (7)
Sheep	Newborn normal	27	168(74–430)	Cunningham (39)
Sheep	Newborn copper deficient	29	13(4–34)	Cunningham (39)
Sheep	Mature (aged) normal diet	44	599(186–1374)	Cunningham (39)
Sheep	Mature (aged) copper deficient	35	27(7–106)	Cunningham (39)

[a] Measured on the dry basis

TABLL 10 (*continued*)

Species	Age and treatment	Number of animals	Copper concentration (p p m)[a]	Reference
Cattle	Newborn normal	41	391(143–655)	Cunningham (39)
Cattle	Newborn copper deficient	20	55(8–109)[b]	Cunningham (39)
Cattle	Mature normal diet	23	200(23–409)	Cunningham (39)
Cattle	Mature copper deficient	41	11.5(2.9–32)	Cunningham (39)
Horse	Mature normal	6	14.8 ± 1.1(12–19)	Beck (7)
Kangaroo (wallaby)	Mature normal	35	17.0 ± 0.38(14–21)	Beck (7)
Domestic fowl	Mature normal	51	14.8 ± 0.42(10–31)	Beck (7)
Domestic duck	Mature normal	34	153 ± 20(37–555)	Beck (7)
Domestic turkey	Mature normal	6	13.5 ± 0.22(13–14)	Beck (7)
Frog (*Hyla aurea*)	Normal	5	292 ± 59(172–454)	Beck (7)
Whale humpback (*Megaptera nodosa*)	Mature normal	37	21.0 ± 0.92(12–38)	Beck (7)
Salmon (*Arripis trutta*)	Mature male	18	15.0 ± 1.21(10–30)[c]	Beck (7)
Salmon (*Arripis trutta*)	Mature female	26	45.2 ± 3.95(20–94)[c]	Beck (7)

[b] Much lower mean levels have been obtained in Western Australia for copper deficient calves

[c] Expressed on dry *fat free* basis

concentration generally occurs vary in different species In man and the rat rabbit, guinea pig dog and cow, *maximum concentrations occur* at or shortly after birth, but in the pig and chick the peak occurs some what earlier in embryonic life (142) During the suckling period the concentration of copper in the liver declines in almost all species al though the total amounts of copper present may, and frequently do rise A notable exception to the general rule of a fall in liver copper con centrations during suckling and until maturity, occurs with the sheep In this species the levels tend to *rise continuously after birth* (102) *In* the rat, also, the peak does not occur until 10 to 15 days after birth although in this case there is a decline thereafter (23)

The functional significance of the congenital liver copper deposits is not completely understood The suggestion that copper is accumulated in fetal liver because the liver is the chief site of hematopoiesis during embryonic life would be easier of acceptance if it were not for the fact that the hematopoietic activity of the liver is at its highest during early embryonic life and has nearly disappeared at birth when in most species, the liver copper concentration is at its peak The more plausible but distinctly teleological suggestion that the liver copper stores of the newborn are provided to compensate for the low copper content of the *maternal milk has received its main support from the well established* fall in liver copper concentrations which occurs during suckling Against this must be set two important facts In the first place the total *amounts* of copper in the liver actually rise in many species during suckling as was pointed out earlier In the second place as was stressed in the case of iron, the total liver stores at birth are normally too small to make more than a very small contribution to the needs of the suckling mammal For instance, the newborn human contains about 2 mg of copper in its liver This amount of copper would be ingested from the milk of the mother within a few days of birth and assuming a 10% absorption of copper from milk it would be absorbed from the milk by the time the baby was a few weeks old The newborn lamb contains an average of 4–5 mg of copper *in its liver an amount which on the same basis* would be obtained from the milk within the first few weeks of the suckling period

c *The Effect of Diet* The principal dietary factors influencing copper storage and hence liver copper concentrations are the levels and proportions of copper molybdenum and *inorganic sulfate* which the diet contains There is evidence also for the existence of other unknown dietary factors affecting copper retention

The liver copper stores of the newborn are greatly reduced by sub

normal copper intakes by the mother This has been demonstrated for several species and is well illustrated by the data of Bennetts and Beck (12) These workers found the liver copper of five ataxic lambs from copper deficient ewes to range from 4 to 8 p p m on the dry basis whereas five normal lambs from healthy ewes grazing pastures adequate in copper gave values ranging from 120 to 350 p p m The liver copper levels of the newborn cannot however be significantly increased by raising the copper intake of the pregnant mother to levels well above normal (Nor as is pointed out later, can the copper content of milk or eggs be raised by feeding extra copper to the normal lactating or laying animal) This failure to raise liver copper levels in the fetus is apparently due to a limited capacity for placental transfer, because post natal copper supplementation of diets already well supplied with copper usually produces very greatly increased liver copper concentrations

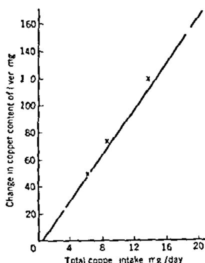


Fig 5 Changes in copper content of the liver of sheep with increasing copper intake (Dick 45)

(Fig 5) These increases can be so high that they reveal a remarkable storage capacity of the liver for copper On the other hand the reduction in the copper content of the liver of the newborn resulting from subnormal intakes by the mother is paralleled by the effects of copper deficient diets on older animals Low liver copper levels have been found in rats and pigs suffering from milk anemia (122 143) in copper deficient dogs (2) and in sheep and cattle grazing copper deficient pastures (12 39 100) as shown in Table 10

In 1945 Dick and Bull (47) first showed experimentally that the storage of copper in the livers of sheep and cattle could be reduced significantly by an increase in the molybdenum intake This finding has

been amply confirmed by Dick (44–46) (Fig 6), by Cunningham (37), and others (42), but some workers were unable to confirm the original results of Dick and Bull and various conflicting and anomalous observations have been made which indicate that the inhibiting effect of molybdenum on copper storage is a complex process involving dietary components other than copper and molybdenum (62, 84, 97, 133) The outstanding investigations of Dick (44–46) have revealed clearly that the level of inorganic sulfate in the diet is of major significance in this respect at least in the sheep *Only in the presence of adequate*

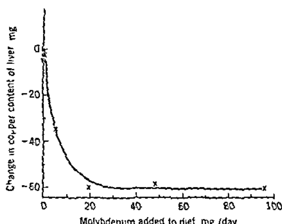


Fig 6 Changes in liver copper storage in the sheep with increasing molybdenum intake (Dick 45)

dietary intakes of inorganic sulfate is the limiting effect of molybdenum on liver copper storage exerted In Dick s experiments it was found that, for a given intake of molybdenum the limitation of copper storage was proportional to the sulfate content of the diet (Fig 7) Much further work is necessary before the exact quantitative relationships among the copper molybdenum and inorganic sulfate intakes can be evaluated but it seems very probable that many of the apparently conflicting reports on the effect of molybdenum on copper storage can be explained on the basis of different levels of inorganic sulfate in the diets employed The whole problem of copper molybdenum inorganic sulfate interrelationships is discussed more fully in the sections dealing with copper and molybdenum absorption and excretion

d *Disease* Disease conditions other than those associated with copper deficiency and molybdenum excess, which reduce the levels of copper in the liver are rare or unknown Abnormally high liver copper levels, on the other hand are characteristic of a number of diseases both of man and of farm animals, apart from those due to excessive copper intakes In man examples of such diseases are Mediterranean anemia hemochromatosis Wilson Uzman s disease (hepatolenticular degenera

tion) cirrhosis and yellow atrophy of the liver, tuberculosis carcinoma and severe chronic diseases accompanied by anemia (26) In sheep a condition of hemolytic jaundice, or yellows" due to chronic copper poisoning has been reported in which liver copper levels as high as 1000 p p m on the dry fat free basis occur This problem is discussed in the section on copper toxicity

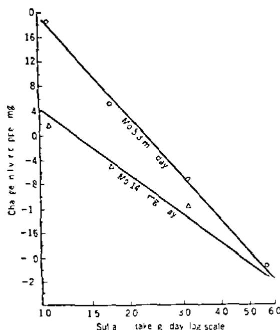


Fig 7 The relation of the level of sulfate intake to the effect of molybdenum upon liver copper storage in the sheep (Dick 45)

4 *Copper in Eye Tissues*

Unusually high concentrations of copper (and of zinc) occur in the eye tissues especially the pigmented portions of a wide range of species including sheep cattle rabbits whales fish and frogs (135) Substantial species differences exist in the magnitude of these concentrations but in all of them the different eye tissues can be placed in roughly the same descending order of copper concentration as follows iris choroid vitreous humor aqueous humor retina (minus pigment epithelium) optic nerve cornea sclera lens Values as high as 105 p p m and 88 p p m of copper on the dry basis have been found for the iris and choroid respectively of the eyes of fresh water trout and of 50 p p m and 13 5 p p m for these tissues in sheep s eyes The levels appear to be somewhat lower in other mammals The copper has been shown to be associated with the pigments (melanins) in the tissues and to be largely bound in nonionic form to protein The function of these copper accumulations in pigmented tissues is unknown but their presence can

not be explained by the presence of free tyrosinase It seems reasonable to suggest that the high concentrations of copper, and other metals (especially zinc) in pigmented eye tissue are concerned with the development or maintenance of natural coloration but there is as yet, no direct evidence for this This question is considered further in relation to the zinc content of these tissues

5 *Copper in Milk*

The concentration of copper in milk is influenced by three main factors These are (1) the species of animal (2) the stage of lactation, and (3) the level of copper intake by the animal There is also considerable individual variation within species even when the second and third factors are comparable In many of the early values for copper in milk analytical errors and contamination also undoubtedly contributed to the great variation in values reported by different investigators but the importance of stage of lactation seems frequently to have been overlooked

Rat s milk is very much richer in copper than that of any other species so far examined Cox and Mueller (35) obtained the extremely high value of 7 mg copper per liter for pooled samples taken from four different stages of lactation compared with 0 6 mg for cow s milk and a quoted figure of 0 5 mg for human milk Most workers have found human milk to be richer in copper than cow s, ewe s, goat s, or mare s milk at similar stages of lactation (4 7, 20) Significant differences in the copper content of the milk of these latter species have not been established and it seems doubtful if they exist

Colostrum is significantly richer in copper than normal milk in all species so far studied An average concentration of copper very close to 0 5 mg /kg has been reported for cow s colostrum (89) Also in the early stages of lactation the true milk of cows, ewes, mares and women is significantly higher in copper than it is in the later stages of lactation Beck (4) found the milk of normal ewes to fall progressively from 0 20–0 64 mg copper per liter in early lactation to 0 04–0 16 mg several months later Similar falls were observed in the milk of some cows but not in others Most of the values lay between 0 05 and 0 20 mg copper per liter Itzerott (73) obtained a very similar range of values namely 0 05–0 14 p p m (mean 0 07) for cow s milk Beck (7) examined the milk of 8 mares and found the copper content of the true milk to fall from a mean of 0 36 mg /l in the first week of lactation to a mean of 0 17 mg /l 4 to 6 weeks later

Beck also found the milk of women to fall steadily from 0 62–0 89 mg

copper per liter in the first few weeks of lactation to 0 15–0 17 mg copper per liter 6 to 7 months later Lesne and co workers (82) showed that in 4 out of 5 women studied the levels fell from 1 05–0 89 mg copper per liter in the first few weeks to 0 60–0 26 mg at the end of lactation They claimed also that there is a seasonal variation in the copper content of cow s and goat s milk related to the type of diet consumed but there appears to be no support for this claim from elsewhere

The addition of copper to diets already adequately supplied with this element has no effect on the copper content of milk (85) The position is similar in this respect to that of iron but in great contrast to that of zinc manganese iodine and cobalt Levels in the milk well above normal can readily be achieved by dietary supplements of these latter elements as will be shown Under conditions of deficient copper intake on the other hand both cows and ewes produce milk subnormal in copper content Beck (4) found the milk of cows and ewes grazing severely copper deficient pastures to fall as low as 0 01–0 02 mg copper per liter In all cases the concentration of copper in the blood was higher than that in the milk This again contrasts with the position with most trace minerals with the exception of iron

Interest in the copper content of milk has stemmed from two sources—its value as a dietary source of copper especially to the suckling and its significance in relation to the development of rancidity or "off flavors" Milk has been recognized as a poor source of copper in the diet since the original demonstration of copper deficiency in young rats fed a diet of cow s milk plus purified iron Milk in fact has formed the basis of almost all diets used in the study of copper deficiency in laboratory animals This refers to milk taken directly from the animal and kept free from metallic contamination Treatment after milking e g pasteurizing drying or holding in metal vessels can result in a variable and *often substantial contamination with copper giving copper concentra* tions more than double that of the original milk (41) If the copper content of cow s milk exceeds about 1 5 p p m a rancid tallowy taste rapidly develops due to catalytic acceleration by the copper of the normal process of fat oxidation in the presence of air Much lower maximum amounts of copper than these of the order of 0 2–0 5 p p m however are compatible with good keeping quality (73) Milk is now usually held and treated in glass and stainless steel containers so that contamination with copper is much smaller than it was in the earlier days of processing

III Copper in Blood

The concentration of copper in the whole blood serum, and plasma of a range of species has been determined in health and disease In recent years, also, a good deal has been learned of the forms in which copper exists in blood and its distribution among the various blood components

1 *Forms of Copper in Blood*

In 1938 Mann and Keilin (93) isolated a copper protein compound from the red cells of cattle They named it hemocuprein and showed it to be a bluish pigment containing 0 34% copper in the cupric form with a molecular weight of 35 000 and 2 copper atoms per molecule No function has yet been found for this compound Whether hemocuprein comprises the whole of the copper in red cells under all circumstances is not known Under certain conditions however, notably following the administration of massive doses of copper to animals red cell copper has been shown to increase to remarkably high levels (26) Such copper could not be removed by repeated washings with saline and it seems likely that it was present as hemocuprein or some similar copper protein compound

In human blood plasma 90% or more of the copper exists in the form of a blue copper protein compound, named ceruloplasmin (69, 70) This compound has been identified as an α globulin with a molecular weight of about 151 000 and containing 8 atoms of copper The relation of ceruloplasmin to hemocuprein is not entirely clear but it has been suggested, since hemocuprein contains 2 atoms of copper in a molecule weighing about 35 000 that it contains 4 units of hemocuprein Ceruplasmin, or some similar compound in which the copper is firmly bound to protein and does not react directly with diethyldithiocarbamate comprises also a very high proportion of the plasma copper of normal rats and dogs and more than half of the total plasma copper of pigs (143) Information on its possible occurrence in the blood plasma of the larger farm animals is badly needed

The remainder of the plasma copper which in man the rat and the dog normally constitutes only a very small proportion of the total exists in plasma in a form which reacts directly with diethyldithiocarbamate This copper since it is mainly nondialyzable, is probably loosely bound to a protein such as albumin the metal binding β globulin (siderophilin) or other proteins (143) Cohn (31) has shown that siderophilin is capable of uniting reversibly with copper without affecting its iron binding capacity and it is well known that albumins are capable of loosely

binding copper *in vitro* but whether siderophilin or one or more of the other plasma proteins act as transport proteins for copper remains to be determined

It is perhaps appropriate at this point to draw attention to the striking difference between serum iron and serum copper although both occur in very similar concentrations Serum copper consists very largely, at least in several species of the functionally active compound ceruloplasmin which Holmberg and Laurell (70) have shown to be a true oxidase classified as a laccase It is normally entirely bound to copper and none is free like siderophilin to take up additional copper Moreover after the oral or intravenous administration of a massive amount of this metal the additional copper is not present as ceruloplasmin but as "direct reacting copper less firmly bound to protein It seems reasonable to suggest that it is this copper which it must be stressed again normally comprises only a small fraction of the total plasma copper which represents transport copper i e copper in passive transit from one site to another By contrast, serum iron consists entirely of iron in passive transport wholly bound to a specific protein the sole or primary function of which is to transport iron Only about one third of this protein is normally bound to iron leaving a considerable reserve capacity

2 *Distribution of Copper in Blood*

The copper in blood is normally distributed approximately equally between cells and plasma This has been demonstrated for man (26 112) and for sheep and cattle (46 51) by direct copper determinations on whole blood and on serum or plasma from which the corpuscular copper is obtained by calculation These determinations invariably give similar values under normal circumstances with a tendency for plasma values to be slightly higher and red cell values slightly lower than those of whole blood Using a highly accurate direct method Lahey and coworkers (79 143) have found the copper content of the erythrocytes to be significantly lower in men and women than that of the plasma (Table 11) It was calculated that the amount of copper in the average red cell (mean corpuscular copper) is about $65 \pm 10\ 8$ $\mu\mu\mu$g and that the concentration of copper in the red cell is normally about 0 000075% The copper content of leucocytes and platelets is considerably less or about one quarter of that of erythrocytes It is obvious from the very much smaller proportion of these cells that failure to separate the leucocytes and platelets from the erythrocytes results in an insignificant error and that they hold an insignificant proportion of the total blood copper

TABLE 11 (26)

DISTRIBUTION OF COPPER AMONG HUMAN WHOLE BLOOD PLASMA AND CELLS

Sex	Number of subjects	Whole blood copper μg /100 ml	Plasma copper μg /100 ml	Cell copper μg /100 ml	Ratio of cell Cu to plasma Cu	Mean corpuscular copper $\mu\mu\mu g$
Male	12	91 5 ± 2 62	105 5 ± 5 03	76 5 ± 2 75	0 75 ± 051	66 ± 7 9
Female	11	96 5 ± 3 54	114 0 ± 4 67	74 3 ± 4 49	0 65 ± 041	64 ± 4 2
Total	23	93 9 ± 2 20	109 5 ± 3 50	75 4 ± 2 71	0 70 ± 0 33	65 ± 2 2

A distribution of the type illustrated in Table 11 applies only to healthy humans under normal conditions and not necessarily to other species and certainly not to all conditions in humans. This is of some importance in view of the tendency to use whole blood copper and plasma copper determinations indiscriminately in the study of copper deficiency and excess in various species. Already there is evidence that plasma copper is much more labile than corpuscular copper. In human pregnancy, for instance, the copper content of the red cells remains normal, whereas that of the serum rises markedly. Also, under conditions of copper deficiency in pigs, the reduction in the copper content of the plasma is very much greater than the reduction in the copper content of the cells (143). Plasma copper, on present evidence, would therefore appear to be a more useful and sensitive indicator of changes in the copper status of an animal than whole blood copper.

3. *Normal Levels of Copper in Blood*

The normal range of concentration of copper in the blood of healthy animals is wide but very similar in all the higher mammals. The normal range for man, pigs, rats, dogs, guinea pigs, sheep, cattle and whales may be set at about 50–180 μg per 100 ml, but a high proportion of the values lies between 80 and 120 μg per 100 ml, with an over-all mean not very far from 100 μg per 100 ml or 1 mg/l (Table 12). A narrower range and significantly lower mean values for whole blood copper of the order of half the levels just given for the higher mammals have been found by Beck (7) for marsupials (kangaroos and wallabies), birds (domestic fowls, ducks and turkeys), fish (salmon and herring) and frogs (Table 12).

Most of the variation within species is due to differences between individuals rather than to day to day fluctuations in the same individual. Thus in a total of 128 normal men and women a range of 68–161 μg per 100 ml of plasma copper was found by Cartwright (26), but in the same individual the values for whole blood, as well as plasma, were found to be fairly constant from day to day and from week to week. The fluctuation was less than ± 30%. Heilmeyer and co-workers (68) and Vallee (140) obtained similar results, but Nielsen (109), in a study of 5 normal women at intervals of 2 to 3 days, found appreciable variation from time to time. There appears, however, to be no cyclic pattern of variation, and changes in plasma copper during the menstrual cycle are within the physiological variation of ± 30% (35, 36).

A small, statistically unconvincing, diurnal variation in both plasma and cell copper has been demonstrated in men and women but no such

variation has yet been shown for other species, or by Vallee (140) in man Both Nielsen (109) and Lahey *et al* (79) found the lowest values in the early morning These values rose during the day and reached their peak in the evening The smallness of this diurnal variation, compared with that of serum iron and its doubtful significance, must be stressed No tendency for an increase in the plasma copper following meals, nor for a decrease during fasting has been observed Physical exertion in man, according to Heilmeyer also produces no change but in sheep violent exercise induces a significant increase in whole blood copper (46) Plasma copper is, however, significantly higher in human females than in males (Table 11) There appears to be no such sex difference in other species

4 *Factors Influencing the Level of Copper in Blood*

The most important factors influencing the concentration of copper in blood apart from the species differences mentioned earlier, are diet and disease In humans pregnancy is a further factor of importance

a *Pregnancy* In 1928 Krebs (77) discovered that serum copper is increased in pregnancy in women This has since been confirmed by many investigators The concentration of copper in the red cells remains at normal levels throughout pregnancy but the plasma copper increases in early pregnancy and continues to rise to reach levels at full term 2 to 3 times those of nonpregnant subjects Normal values reappear during the first few weeks post partum Thus Nielsen (109), in a study of 31 pregnant women found the serum copper to increase from the third month of pregnancy and to reach an average level at delivery of 269 μg per 100 ml compared with a normal nonpregnant level which is given by him as 123 μg per 100 ml Blood taken from the umbilical cord of 20 infants gave the very much lower figure of 56 μg per 100 ml Rottger (112) found the serum copper of women to rise in pregnancy from 100 to 280 μg per 100 ml and the red cell copper to remain normal throughout at 120 μg The red cells of newborn infants were found to contain similar levels of copper to those of the mother but the serum copper was very much lower (54 μg per 100 ml), as in Nielsen s study Fay and co workers (57) obtained essentially similar results to those of Nielsen and Rottger Serum from the umbilical vein of 14 newborn infants averaged 75 $\pm$ 14 μg copper per 100 ml and the maternal serum copper was 260 $\pm$ 42 μg per 100 ml By the second week of life the values were found to reach adult levels and to remain at this level throughout childhood

No fully satisfactory explanation of these changes has been put for

TABLE 12

THE COPPER CONTENT OF THE BLOOD OF DIFFERENT SPECIES

Species	Age and condition	Copper concentration[a]		Reference
		Mean	Range	
Man	Mature healthy	1 14[b]	—	R A Kehoe *et al* (74a)
Man	Mature healthy	1 06[c]	0 7–1 40	Heilmeyer *et al* (68)
Man	Healthy adult male	1 10 ± 0 12[c]	—	Nielsen (109)
Man	Healthy adult female	1 23 ± 0 16[c]	—	Nielsen (109)
Man	Pregnant female at delivery	2 69 ± 0 49[c]	—	Nielsen (109)
Man	Healthy female late pregnancy	2 30	—	*Rottger* (112)
Man	Healthy adult female	1 00[c]	—	Rottger (112)
Sheep	Healthy mature	1 01 ± 0 96[b]	0 75–1 35[b]	Beck (7)
Sheep	Healthy mature	0 91[b]	—	Cunningham (39)
Sheep	Copper deficient	0 37[b]	—	Cunningham (39)
Sheep	Copper deficient	—	0 1–0 5[b]	Bennetts and Beck (12)
Cow	Healthy mature	—	0 7–1 7[b]	Beck (5)
Cow	Healthy mature	0 93[b]	—	Cunningham (39)
Cow	Copper deficient	0 53[b]	—	Cunningham (39)
Pig	Healthy mature	—	1 6–1 8[b]	Kehoe *et al* (74a)
Pig	Healthy young	—	1 5–1 7[b]	Kehoe *et al* (74a)
Guinea pig	Healthy mature	0 50 ± 0 006[b]	0 40–0 58[b]	Beck (7)
Domestic fowl	Healthy mature	0 23 ± 0 008[b]	0 11–0 47[b]	Beck (7)
Domestic duck	Healthy mature	0 35 ± 0 007[b]	0 22–0 45[b]	Beck (7)
Domestic turkey	Healthy mature	0 23 ± 0 007[b]	0 18–0 28[b]	Beck (7)
Kangaroo (wallaby)	Healthy mature	0 34 ± 0 021[b]	0 27–0 39[b]	Beck (7)
Frog (*Bufo marinus*)	Healthy mature	0 46 ± 0 04[b]	0 25–0 67[b]	Beck (7)
Salmon (*Arripis trutta*)	Healthy mature	0 58 ± 0 02[b]	0 45–0 64[b]	Beck (7)

[a] Measured in μg Cu per ml of whole blood or serum

[b] Whole blood

[c] Serum

ward, although it has been suggested (116) that the high serum copper levels of pregnancy act as a hemopoietic stimulus related to the physiological anemia of pregnancy in women In the sheep which is the only other species for which detailed data on this point appear to be available there are no changes in the copper content of whole blood characteristic of pregnancy (5, 50) The blood of the newborn lamb, however, like that of the newborn human, is significantly lower in copper than that of its mother and there is a similar rapid post partum rise to adult levels (50)

b *Diet* Whole blood and plasma copper levels can be steadily reduced below normal by the feeding of diets deficient in copper Hypocupremia has been demonstrated in rats pigs, and dogs suffering from experimental milk anemia (2 80, 123) in prolonged anemia of children (81) and in sheep and cattle grazing on natural copper deficient pastures (12 48) Values as low as 10 and 20 μg copper per 100 ml of whole blood have been reported in sheep and cattle under such conditions and as low as 19 μg per 100 ml in experimentally induced copper deficiency in pigs (80) In this latter study with pigs plasma and red cell copper were also determined with results as set out in Table 13

On the other hand, the addition of moderate amounts of copper to diets already well supplied with this element does not necessarily result in consistently higher than normal blood copper levels This is in contrast to the large increase in the liver copper levels which occurs under these conditions Definite hypercupremia results, however, from higher levels of copper intake and from single massive doses or injections (19, 38 49) In the latter case the hypercupremia is only transitory

The inhibiting effect of molybdenum on liver copper storage is paralleled, under certain conditions by its effect on blood copper levels Hypocupremia has been reported in sheep and cattle grazing under conditions of copper sufficiency, but with added molybdenum, by Cunningham (37) Marston (94), on the other hand, reports that in sheep added molybdenum at the rate of 50 mg/day, did not materially influence the concentration of copper in the blood under conditions of copper sufficiency and tended to maintain this concentration on copper deficient grazing Essentially similar results to those of Marston were reported for sheep and cattle by Green (62) Dick (44) has shown the concentration of copper in the blood of sheep to be raised by very high intakes of molybdenum when the diet also contains inorganic sulfate It seems that molybdenum can either raise or lower blood copper levels depending upon its level of intake and the proportion of inorganic sulfate in the diet

TABLE 13

COPPER CONCENTRATIONS IN THE BLOOD OF NORMAL AND COPPER DEFICIENT PIGS (80)

	Number of animals	Age in days	Whole blood copper µg/100 ml	Plasma copper µg/100 ml	Red blood cell copper µg/100 ml	Mean corpuscular copper µµµg
Copper deficient	30	84 ± 10.9	19 ± 9.3	15 ± 7.0	67 ± 28.4	26
Normal	10	84 ± 19.6	138 ± 15.2	186 ± 15.4	110 ± 42.0	61

c *Disease* Diseases associated with hypocupremia other than those of nutritional origin, are very uncommon None has been reported in farm animals and only two in man These are the nephrotic syndrome and Wilson Uzman s disease both of which are accompanied by increased urinary excretion of copper The mean plasma copper level in 16 patients with nephrosis was recently found to be 64 ± 20 μg per 100 ml, compared with 116 ± 14 μg per 100 ml in normal subjects (27) The mean serum copper of 17 patients with Wilson Uzman s disease has been reported as 60 ± 15 4 μg per 100 ml, compared with 108 ± 9 8 for 12 normals (3) It is of great interest that in this investigation markedly reduced levels of the serum copper protein ceruloplasmin were observed They constituted the most specific biochemical abnormality found

Hypercupremia occurs much more frequently (26, 79 140) In man an increase in plasma copper has been observed in most acute and chronic infections This increase usually commences soon after the onset of the infection and returns to normal during convalescence Hypercupremia also occurs in chronic and acute leukemia Hodgkin s disease, various types of anemia collagen disorders, hemochromatosis and certain other disorders (Table 14) including myocardial infarction (140) In hyperthyroidism the increase in plasma copper is accompanied by a decrease in red cell copper The mechanism underlying these changes is not yet understood although following experiments in rats in which inflammation was produced by the injection of turpentine and certain other agents a tentative suggestion has been put forward that the intestinal barrier to copper is lowered leading to its rapid absorption and passage to the liver where more ceruloplasmin is formed (79)

IV Absorption and Excretion

1 *Absorption*

Nothing is known of the mechanism of absorption of copper and the factors influencing its absorption are very imperfectly understood It is strange that so little has been done on this phase of copper metabolism in man in view of the relationship of this element to iron and the great amount of work which has been done on iron absorption

Studies in dogs (117) indicate that copper is absorbed in the upper jejunal loops but not at all in the middle and distal loops Tompsett (138) states that absorption of copper probably occurs in man in the upper part of the small intestine where the contents still have a pronounced acid reaction He points out that the reaction of the intestinal contents must exert a very marked influence on copper absorption and

TABLE 14

BLOOD COPPER IN VARIOUS CLINICAL CONDITIONS IN HUMANS (143)

Condition	Number of subjects	Whole blood copper (μg %)	Plasma copper (μg %)	Cell copper (μg %)	Volume of packed red blood cells (ml per 100 ml)	Plasma iron (μg %)
Normal	63	98 ± 13	109 ± 17	115 ± 22	47	115 ± 42
Pregnancy	30	169	222	130	37	91
Infection	37	141	167	116	41	57
Acute leukemia	19	195	236	98	27	171
Chronic leukemia	21	119	148	101	39	113
Hodgkin's disease	14	142	171	109	40	78
Pernicious anemia	10	111	121	98	27	173
Aplastic anemia	8	130	152	86	28	203
Iron deficiency anemia						
Adults	9	114	132	109	30	26
Infants	24	155	168	152	28	31
Hemochromatosis	*14*	*103*	*134*	—	—	234
Hyperthyroidism	7	108	140	70	46	124
Hypothyroidism	7	105	126	103	37	83
Wilson's disease	3	79	55	110	42	64
Nephrosis	3	70	80	119	44	62

that this in turn must depend upon a number of factors such as the gastric acidity, the base (especially calcium) content of the diet, and the character of the intestinal secretions

These suggestions do not seem to have been followed up in any very thorough way but the critical investigations of Dick (44–46) on the assimilation and storage of copper in the sheep have convincingly revealed the profound influence of diet upon copper absorption and excretion in this species Additions of large amounts of calcium carbonate and ferrous sulfide to the diet markedly reduce the assimilation of copper by these animals Neither elemental sulfur nor sodium thiosulfate is effective in this way It is argued that both the calcium carbonate and the ferrous sulfide act by reducing the solubility of copper—the former by a lowering of intestinal acidity, as suggested by Tompsett and the latter by the formation of insoluble copper sulfide

More difficult to explain but of more fundamental importance are the effects of a relatively high dietary intake of molybdenum in limiting copper assimilation and storage This effect of molybdenum is only exerted as indicated previously, in the presence of a sufficient quantity of inorganic sulfate in the diet Salts of zinc, nickel, and iron, within the limits fed have no such effect on copper accumulation in the liver, and hence by implication on copper absorption Similar findings in regard to molybdenum in both sheep and cattle have been made by Cunningham (37) but a range of other elements including zinc, manganese tungsten vanadium chromium rhenium, uranium, and tantalum, when similarly fed to sheep had no such limiting effects In rats also molybdenum has been shown to reduce copper absorption greatly (33) Much more information will be required before the full quantitative relationships among copper molybdenum and inorganic sulfate intakes can be expressed or a satisfactory explanation given of how these dietary constituents function Nevertheless the evidence already accumulated provides a striking example of trace element interrelationships of great practical and fundamental importance in nutrition

It is important to appreciate the fact that the studies of the influence of molybdenum on copper metabolism have utilized the extent of copper retention or storage in the liver as the main criterion upon which any effect on absorption is judged Reduced copper retention could be the over all result of two distinct processes—lowered absorption of ingested copper and increased excretion of copper already absorbed and stored There is evidence that under conditions of high molybdenum and sulfate intake both these processes are affected i e not only is the assimilation and storage of ingested copper reduced but the rate of copper loss is

increased (45) Supplements of calcium carbonate and ferrous sulfide on the other hand appear merely to reduce the proportion of the ingested copper which is assimilated and retained by the animal

It is further apparent from the wide variations in the reaction of individual animals kept under identical conditions and the incidence of copper deficiency symptoms in sheep and cattle grazing certain pastures "normal" in both copper and molybdenum that there are still unrecognized factors which influence copper absorption The influence of high intakes of zinc on either the absorption or utilization of copper, or on both has been demonstrated in rats The resulting anemia in these animals can be overcome by dietary additions of copper This question is considered more fully in the section on zinc toxicity in Chapter 7 Whether zinc similarly affects copper metabolism in grazing ruminants is not known but it is obvious that this and other elements warrant further investigation in relation to copper deficiency conditions in these species

It is possible also that metabolic changes in plants cause copper to enter combinations from which it cannot readily be released and assimilated by animals At present very little is known of the chemical forms in which copper occurs in plants and even less of the relative availability of different known forms of copper in organic or inorganic combination A valuable and highly interesting beginning in the investigation of this aspect of the nutritional physiology of copper has been made by Mills (106) This worker showed that the greater part of the copper in herbage exists in a bound form and that much of this copper is not extractable by organic solvents or by dilute aqueous solutions of organic chelating agents It was found further that seasonal variations in the solubility of herbage copper exist and that rather less water soluble copper is present in pastures in which "swayback" in lambs occurs than in normal herbage Mills has also obtained evidence that a stable water soluble copper complex present in herbage is more readily utilized by the copper deficient rat than are cupric ions The nutritional significance of these findings is unknown but they point clearly to the importance of the chemical combinations in which copper can occur naturally and to the need for further research along the lines initiated by Mills

The relative inability of sheep to assimilate copper as copper sulfide has already been mentioned Many years earlier it was shown that anemic rats are also unable to use the copper of copper sulfide or copper porphyrin (124) The oxide hydroxide iodide glutamate glycerophosphate cystein cuprous mercaptide aspartate citrate nucleinate and

pyrophosphate on the other hand were found to be readily utilized as a source of copper by such animals (124)

Several investigations with radioactive copper have been made of the absorption, distribution in the blood and tissues, and excretion of this element in man, rats guinea pigs dogs, rabbits, and cattle (32 118) These have revealed some species differences particularly in the distribution of the absorbed metal but they have all served to emphasize the poor absorption of orally administered copper In cattle 75% of the radioactive copper administered in this way was excreted in the feces in the first 5 days and about 3% in the urine whereas in dogs 80% was found in the feces in the first 3 days and only traces in the urine Studies with stable copper have similarly demonstrated the very poor absorption of dietary copper Only about 5% of the copper in ordinary diets over a wide range of copper intakes appears normally to be absorbed and retained by the animal body An exception to this generalization occurs in Wilson Uzman s disease of man in which very much higher absorption of copper has been observed

2 *Excretion*

Many balance experiments with stable copper, and the studies with radioactive copper just cited have shown conclusively that the chief channel of excretion of copper in all species is the intestinal tract Under ordinary dietary conditions 90% or more of the ingested copper appears in the feces Most of this consists of unabsorbed copper but active excretion via the bile and possibly directly through the intestinal wall has been demonstrated in man The bile fluid is relatively rich in copper and van Ravesteyn (111) observed its content to rise temporarily above the normal range after an intravenous injection of copper Within a few weeks most of the injected copper appeared in the feces The importance of the bile as a channel for the excretion of copper is indicated further by the finding that in patients with liver cirrhosis accompanied by biliary obstruction there is an elevation in urinary copper whereas patients with classical nutritional Laennec s cirrhosis without significant biliary obstruction have normal or only slightly elevated serum and urinary copper (3)

Oral administration of copper sulfate does not result in any increase in urinary copper in man and intravenous injection is followed by only a slight transient rise (111) Furthermore copper balance studies in man have revealed no apparent correlation between the amount of copper ingested and the amount excreted in the urine daily Considerable individual variation in the amount of copper excreted daily in the urine has been reported but the amount is normally extremely small Porter

(109a) was unable to detect any urinary copper in 5 out of 11 normal adults In 2 of these subjects the total was less than 3 5 μg copper daily and in 4 it ranged from 3 5 to 14 7 μg daily Beck (7) found the urinary copper excretion of 2 normal adult males to be 14 μg and 22 μg daily It is obvious that the kidneys are not an important route for the excretion of copper in normal individuals

In Wilson Uzman s disease excessive urinary excretion of copper which may reach 1500 μg/day is a constant feature of the condition Amino acid excretion is also very high in this disease Increasing the amino acid excretion by raising the protein intake or by the administration of cortisone has been shown by Bearn and Kunkel (3) to result in a parallel increase in the urinary excretion of copper These workers suggest that the increased absorption of copper which characterizes Wilson Uzman s disease and the resulting accumulation of copper in the tissues may give rise to liver cirrhosis and renal tubular damage causing increased excretion of a number of substances through the lowered renal threshold Although amino acid excretion and copper excretion in the urine show parallel increases there is no evidence that the excretion takes place in the form of copper amino acid complexes In nephrosis it appears that the excess urinary copper which characterizes this condition occurs as a nondialyzable copper protein complex at least partly in the form of ceruloplasmin itself (27) This compound is markedly reduced in the plasma of nephrotic patients and the amount of copper excreted in their urine has been correlated with the amount of urinary protein (27)

It is pertinent to mention at this point that excessive urinary excretion of iron and zinc as well as copper occurs in nephrosis accompanying the proteinuria The little which is known about zinc in this connection is discussed in the section on zinc excretion in Chapter 7 The iron in the urine of nephrotic patients is like copper nondialyzable and can be correlated with the amount of urinary protein present It is suggested that it occurs largely as transferrin since there is a highly significant reduction in the concentration of this compound in the plasma of nephrotics (27) These findings emphasize the firmness of the protein binding of these metals in the body

Injected copper appears to be much less readily excreted than orally administered copper Thus Comar (32) found with cattle that only 6% of a single dose of intravenously injected radioactive copper was excreted in the first 5 days compared with 78% of a rather similar oral dose Moreover the small amount of injected copper which was excreted was distributed about equally between the urine and feces Somewhat similar results were obtained by Schubert and Riezler (118) with dogs

These findings explain the long period of therapeutic efficiency of injected copper in cattle and sheep (32 67) It has been shown that 200 mg of copper, as the sulfate, injected intramuscularly, can protect copper deficient dairy cows for at least 5 months and a single injection of 20 mg of copper into copper deficient ewes during pregnancy has been found effective in the prevention of ataxia in the lambs

When considering dietary factors influencing copper storage, and again in the preceding section dealing with copper absorption mention was made of the profound influence of molybdenum on copper retention in the presence of adequate dietary sulfate This question is discussed further in Chapter 4 Dick (44–46) has adduced evidence that molybdenum under these conditions limits copper retention in the sheep both by reducing absorption of this element and by increasing its excretion Copper balance studies to provide direct support for this claim and to throw some light on the mechanism of the molybdenum plus sulfate effect on excretion are needed in the sheep and in other species

Additional sources of loss of copper from the body occur in women between the menarche and menopause in the menstrual blood and in all species as a result of reproduction and lactation None of these appears to impose as great a drain upon dietary or body copper as does loss of iron by these means upon exogenous and endogenous sources of this element (see Chapter 2) Copper loss through normal menstrual flow is small It averages about 0 5 mg per period (83) or slightly less than 0 02 mg [(0 5 × 13)/365] per day This is one twenty fifth of the average loss of iron per day through menstruation whereas the intake of copper in the average diet is of the order of one sixth of that of iron The loss of copper in the milk at the height of lactation is very much higher than this (of the order of 0 4 mg /day) but there is no evidence that this imposes a serious strain on the individual

V Manifestations of Deficiency and Functions of Copper

Copper is remarkable among the trace elements for the number and variety of functions which it serves in the animal body This is indicated by the range of clinical conditions which develop in different species and even within the same species as a result of a dietary deficiency of copper Anemia is general but depressed growth bone disorders depigmentation of hair fur or wool and abnormal wool growth demyelination of the spinal cord fibrosis of the myocardium and gastrointestinal disturbances (diarrhea) have all been observed in copper deficient animals of one or more species and all have been shown to be

prevented or alleviated by the administration of suitable amounts of copper

The extent to which one or more of these symptoms is revealed depends upon the species and the age of the animal and the severity and duration of the copper deficiency As the copper available to the animal becomes insufficient for all the metabolic processes involving copper as a result of inadequate intake and depletion of body reserves certain of these processes fail in the competition for the inadequate supply In any one species there seems to be an ordered sequence of distinct symptoms as first one and then another metabolic process becomes adversely affected by the lack of copper This sequence of events is not the same in all species and in fact, certain of the manifestations of copper deficiency common in one species have never been recorded in others Moreover even within a species the stage of maturity of the animal and the rapidity with which the copper deficiency develops can greatly influence the symptoms actually observed Thus in the sheep the processes of pigmentation and keratinization of the wool are the first to be affected by a lowered copper status and at certain levels of intake no other functions appear to be impaired The particular enzyme systems involved in these processes are clearly more sensitive to lack of copper in this species than for instance in the pig Further in calves and piglets copper deficiency is only very rarely accompanied by ataxia and ataxia has never been conclusively demonstrated in rats or dogs Both young and adult rats have been reared and maintained on a copper deficient diet for several generations without any signs of nerve lesions or any abnormality of the brain or spinal cord (60) Yet this condition occurs readily in lambs from copper deficient ewes Apparently ovine fetal myelin tissue has a special copper requirement compared both with that of other tissues or functions involving copper in other species at this stage of development and with myelin tissue in the same species at later growth stages

Many more unexplained differences in the manifestations of copper deficiency exist which will become apparent as they are described in the following sections Many significant facts have been learned about them but little is yet known of the underlying mechanisms and practically nothing of the precise way in which copper functions at the cellular level More thorough and detailed studies of the enzyme systems of the different tissues involved such as have been attempted in relation to blood formation would seem to be the most fruitful form of attack upon this problem

1 *Copper in Relation to Blood Formation*

Anemia is a characteristic of copper deficiency in all species so far studied These include rats rabbits chickens pigs dogs, sheep goats, cattle and man It is not necessarily however a dominant feature of the deficiency syndrome in all species under all conditions The extent to which anemia develops depends partly upon the species but mainly upon the severity of the copper deficiency and the rate at which it develops and the length of time over which it occurs It is influenced also by the age of the animal and the magnitude of its body copper stores when first restricted to the deficient diet At first the copper deficiency is expressed in a slow depletion of the copper stores in the tissues especially the liver This is followed by a steady fall in the copper concentration of the blood until the level is below that necessary to maintain normal hematopoiesis A blood copper concentration of 20 μg per 100 ml has been suggested as the minimum level at which normal hematopoiesis can take place in the pig (80) and 10–12 μg per 100 ml has been found to limit blood formation in the sheep (5 100) If such low levels in the blood are maintained for any length of time anemia inevitably develops and may progress rapidly to a fatal termination Weanling rats pigs or dogs when placed on a virtually copper free milk diet (with added iron), very quickly develop anemia as the dominant symptom of copper deficiency because of the rapid fall in blood copper to limiting levels

Many attempts have been made to elucidate the manner in which copper functions in erythropoiesis Since copper is not a constituent of the hemoglobin molecule (55) it must be concerned either in the formation and maturation of the red cells or in some aspect of hemoglobin synthesis, such as the incorporation of iron into the molecule or in both processes Present evidence to be discussed below is not consistent for all species but indicates that copper plays an essential role both in the maturation of red cells and in the mobilization and utilization of iron in hemoglobin formation

a *The Nature of the Anemia* The morphological characteristics of the peripheral blood in copper deficiency anemia vary rather widely in different species and with different observers In rats (130) rabbits (131) and pigs (80) the anemia has been described as hypochromic and microcytic indistinguishable from that of iron deficiency (Table 15) The bone marrow in copper deficiency in pigs has also been shown to undergo a normoblastic hyperplasia as in iron deficiency In Australia and New Zealand the anemia accompanying copper deficiency in cattle

TABLE 15

MORPHOLOGICAL CHARACTERISTICS OF BLOOD OF COPPER DEFICIENT AND IRON DEFICIENT PIGS (80)

	Group				
	Control	Copper deficient	Iron deficient	Copper and iron deficient	
Number of animals	10	30	10		4
Age in days	84 ± 19.6	84 ± 10.9	59 ± 1.8		56
RBC (× 10⁶/mm³)	7.6 ± 0.68	5.6 ± 1.34	5.9 ± 1.28	4.4	(3.5–5.7)
Hb (g/%)	14.0 ± 1.03	6.4 ± 1.70	5.9 ± 1.30	3.4	(3.1–4.0)
VPRC (ml per 100 ml)	42 ± 2.8	22 ± 6.2	21 ± 4.5	13	(12–16)
MCV (μ^3)	56 ± 2.9	39 ± 4.9	36 ± 3.1	30	(26–35)
MCH ($\mu\mu g$)	18 ± 1.0	11 ± 1.9	10 ± 1.0	8	(7–9)
MCHC (%)	33 ± 0.8	29 ± 1.9	29 ± 2.9	26	(25–27)
Reticulocytes (%)	5 ± 3.0	1 ± 3.1	9.4 ± 4.4	6.8	(3–9)

VPRC volume of packed red cells
MCV mean corpuscular volume
MCH mean corpuscular hemoglobin
MCHC mean corpuscular hemoglobin concentration

1 *Copper in Relation to Blood Formation*

Anemia is a characteristic of copper deficiency in all species so far studied. These include rats, rabbits, chickens, pigs, dogs, sheep, goats, cattle, and man. It is not necessarily, however, a dominant feature of the deficiency syndrome in all species under all conditions. The extent to which anemia develops depends partly upon the species but mainly upon the severity of the copper deficiency and the rate at which it develops and the length of time over which it occurs. It is influenced also by the age of the animal and the magnitude of its body copper stores when first restricted to the deficient diet. At first the copper deficiency is expressed in a slow depletion of the copper stores in the tissues, especially the liver. This is followed by a steady fall in the copper concentration of the blood until the level is below that necessary to maintain normal hematopoiesis. A blood copper concentration of 20 μg per 100 ml has been suggested as the minimum level at which normal hematopoiesis can take place in the pig (80) and 10–12 μg per 100 ml has been found to limit blood formation in the sheep (5, 100). If such low levels in the blood are maintained for any length of time anemia inevitably develops and may progress rapidly to a fatal termination. Weanling rats, pigs, or dogs, when placed on a virtually copper free milk diet (with added iron), very quickly develop anemia as the dominant symptom of copper deficiency because of the rapid fall in blood copper to limiting levels.

Many attempts have been made to elucidate the manner in which copper functions in erythropoiesis. Since copper is not a constituent of the hemoglobin molecule (55) it must be concerned either in the formation and maturation of the red cells or in some aspect of hemoglobin synthesis, such as the incorporation of iron into the molecule, or in both processes. Present evidence, to be discussed below, is not consistent for all species but indicates that copper plays an essential role both in the maturation of red cells and in the mobilization and utilization of iron in hemoglobin formation.

a *The Nature of the Anemia.* The morphological characteristics of the peripheral blood in copper deficiency anemia vary rather widely in different species and with different observers. In rats (130), rabbits (131), and pigs (80) the anemia has been described as hypochromic and microcytic, indistinguishable from that of iron deficiency (Table 15). The bone marrow in copper deficiency in pigs has also been shown to undergo a normoblastic hyperplasia as in iron deficiency. In Australia and New Zealand the anemia accompanying copper deficiency in cattle

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	Group				
	Control	Copper deficient	Iron deficient	Copper and iron deficient	
Number of animals	10	30	10	4	
Age in days	94 ± 19 6	81 ± 10 9	59 ± 4 8	56	
R B C (× 10^6/mm ^{3})	7 6 ± 0 68	5 6 ± 1 34	5 9 ± 1 28	4 4	(3 5–5 7)
Hb (g /%)	14 0 ± 1 03	6 4 ± 1 70	5 9 ± 1 30	3 4	(3 1–4 0)
V P R C (ml per 100 ml)	42 ± 2 8	22 ± 6 2	21 ± 4 5	13	(12–16)
M C V (μ^3)	56 ± 2 9	39 ± 4 9	36 ± 3 1	30	(26–35)
M C H ($\mu\mu$g)	18 ± 1 0	11 ± 1 9	10 ± 1 0	8	(7–9)
M C H C (%)	33 ± 0 8	29 ± 1 9	28 ± 2 9	26	(25–27)
Reticulocytes (%)	5 ± 3 0	4 ± 3 1	9 4 ± 4 4	6 8	(3–9)

V P R C volume of packed red cells
M C V mean corpuscular volume
M C H mean corpuscular hemoglobin
M C H C mean corpuscular hemoglobin concentration

has been reported to be hypochromic and macrocytic (11 39) and in sheep it has been found to be hypochromic and microcytic in the lambs and macrocytic in the ewes (11, 99) In dogs on the other hand, two groups of workers have shown the anemia of copper deficiency to be normocytic and normochromic (2 90) In contrast to the classical picture of iron deficiency anemia, the anemia in this species is characterized by a reduction in the number of erythrocytes, with the maintenance of relatively normal red cell indices The bone marrow shows no evidence of a deficiency in hemoglobin but rather of a defective maturation of the erythrocytic elements The distribution of cells of the erythrocytic series is shifted decidedly to the left, with a decrease in the proportion of late normoblasts and an increase in the number of more *immature forms with larger nuclei and more basophilic cytoplasm* Furthermore the quantity of cytoplasm and the hemoglobin content of the late normoblasts are comparable with those of normal dogs and greater than those of iron deficient dogs (2)

The similarity of the hematological changes in iron deficiency and copper deficiency in rats, rabbits, and pigs together with other biochemical evidence, has been interpreted as indicating that a common mechanism (lack of available iron) is involved in both conditions The results of the studies with dogs just described, together with evidence from a detailed study of the blood picture in copper deficient cows and ewes suggest that copper plays a role in the maturation of erythrocytes distinct from its function in hemoglobin formation Support for such a role for copper is given not only by earlier work with dogs (90) but also by the observation that some reticulocyte response occurs in milk anemia in rats fed copper alone (but not iron alone) and that such animals respond to copper by an increase in the number of red cells without an increase in hemoglobin concentration (132)

b *Copper and Iron Metabolism* Conclusive evidence that copper functions in the utilization of iron emerged within a few years of the original demonstration of its necessity for blood formation Elvehjem (52) concluded in 1935 that "In the animal body it [copper] is not concerned with the assimilation of iron but with the transformation of the ingested iron into hemoglobin" Schultze (120) five years later stated "Much evidence indicates that copper is not necessary for the absorption and storage of iron in tissues but that it facilitates or is essential for, the utilization of iron by the blood forming organs and for mobilization of iron from the tissues This latter interpretation of the role of copper in iron metabolism and the conception of the anemia

of copper deficiency as in effect an iron deficiency anemia induced by the lack of copper, has come to be somewhat uncritically accepted It receives support from many investigations of copper deficiency in sheep and cattle in which extensive deposits of iron are found in the tissues of copper deficient animals but not from the studies of the morphological characteristics of the anemia in these species described earlier In regard to the former, Marston (95) reports that in sheep the concentration of iron in the liver rose steadily from the normal levels of 200–400 p p m of dry tissue to more than 35 000 p p m in animals which had been confined to copper deficient grazing for a period of two years The value of these observations in copper deficient sheep and cattle would be greatly enhanced by more thorough investigations of iron metabolism including plasma iron determinations

A wider conception of the relation of copper to iron metabolism is put forward by Gubler and co workers (63) on the basis of their detailed and critical studies of copper deficiency in pigs These workers produced additional convincing evidence that in this species copper facilitates mobilization of iron from the tissues and is concerned in the utilization of iron in hemoglobin synthesis *They have also shown that copper favors the absorption of iron from the gastrointestinal tract and were able to demonstrate an impaired ability to absorb iron in copper deficient pigs* This finding is so at variance with accepted views based largely on work with other species that it seems necessary to give the evidence on which their conclusions are based This can be summarized as follows (a) In copper deficient pigs there was a reduction in total body iron (as indicated by the sum of liver kidney spleen heart and blood iron) to levels comparable with those found in iron deficient animals although they were fed the same quantity of iron as the controls (b) Following the oral administration of radioactive iron the total amount of such iron in the tissues was significantly less than in control animals (c) Copper deficient pigs responded to copper only when iron was included in the diet (d) There was a rapid and marked increase in the plasma iron level when iron was given with or following copper administration to pigs deficient in both iron and copper whereas there was no such increase following iron alone In further studies with rats (28) these workers presented evidence that the total body radioiron content of copper deficient animals fed radioiron is lower than that of similar rats given copper in the diet

The fact that copper influences iron metabolism at least in pigs and rats in such diverse sites as the mucosal cell the liver and the bone

marrow, suggests as Gubler (63) states that copper may in *some basic manner, be concerned wherever and whenever iron moves*" Nothing is known of any such mechanism at the present time Knowledge is also lacking of the stage of erythrogenesis at which copper exerts its action It is apparently not necessary for the synthesis of protoporphyrin since normal amounts of free protoporphyrin have been found in the mature erythrocytes of copper deficient pigs and higher than normal amounts of free protoporphyrin in the red cells of copper deficient sheep (34) It seems more likely to be concerned with the incorporation of iron into protoporphyrin to form heme and with the respiration and development of the immature red cells through the intervention of enzymes of the hematin type, especially as the development of the red cells in the closed intersinusoidal capillaries takes place in a medium of low oxygen tension (115) In this connection it is pertinent to mention that an adequate intake of copper has been shown to be essential for the formation and maintenance of normal cytochrome oxidase and catalase activity in certain tissues in the rat The cytochrome oxidase activity of liver, heart and bone marrow, and the catalase activity of erythrocytes liver, and kidney are reduced under conditions of copper deficiency in this species (121) On the other hand Marston (96) could find no such decrease in the cytochrome oxidase status of the tissues of copper deficient sheep and Lahey *et al* (80) failed to observe a significant decrease in the catalase activity of erythrocytes liver and kidney in copper deficient swine Clearly much further study will be required before the exact role of copper in blood formation is revealed

2 *Copper and Bone Development*

Bone defects resulting in spontaneous fractures have been reported in sheep and cattle grazing on copper deficient pastures in certain parts of the world but not in others In parts of Florida cattle are unable to form bone properly without copper supplements with the result that a condition very similar to rickets occurs in young calves and osteoporosis in older animals (42) The abnormality in bone formation is evidenced by broken bones and enlarged joints In Australia and New Zealand the bones of calves and lambs tend to fracture easily both in areas of simple copper deficiency (9 12) and in areas where the copper deficiency is complicated by higher than normal concentrations of molybdenum in the pastures (37) Roentgenographic and histological examinations of the bones have not been carried out but Cunningham (37) states that the fractured bones showed a mild

degree of osteoporosis were almost normal in app thickness and showed no microscopic defect which their brittleness Davis (42) reported that the bone under conditions of normal phosphorus intake and w with changes in blood calcium or phosphorus

Bone changes have not been reported in coppe rabbits but they have been observed in foals (10), the limbs, with excessively flexed hocks and crooked f reported during studies of copper deficiency in p young dogs made severely deficient in copper a gr characterized by abnormally thin cortices deficient tr epiphyses has been demonstrated (2) Fractures and bones occurred in many of these dogs (Fig 8) Ser



Fig 8 Three littermate dogs after three months on pu left is a normal control dog receiving the diet plus addec rough hair and deformed legs of the copper-deficient dogs on the right (Baxter *et al* 2)

phorus and vitamin D levels were found to be nor animals and the bone lesions were not characteristic emphasized that the bone disorder was a specific ef ciency and not a result of anemia Iron deficiency anemia failed to produce similar bone changes

Detailed histological and chemical studies underta

bone showed the ash, calcium phosphorus and carbon dioxide contents to be normal and in fact practically identical in the control and copper deficient dogs (2) The authors conclude that the bone disorder consists of a diffuse osteoporosis with no primary disturbance in the calcification mechanism and suggest that copper may be essential for normal osteoblastic function in the same way as are other nutrients such as ascorbic acid

3 *Copper in Relation to Demyelination of the Nervous System*

A nervous disorder characterized by incoordination of gait has been recognized for many years in lambs born of ewes grazing certain restricted areas in many parts of the world (95 114) Various local descriptive names have been given to the condition such as swayback, swingback, Gingin rickets lamkruis renguera etc but they appear to be pathologically identical maladies to which the general term "enzootic ataxia first proposed by Bennetts or, more properly, enzootic neonatal ataxia could appropriately be applied Enzootic neonatal ataxia is overwhelmingly a problem of lambs but it has been reported to occur occasionally in goat kids (119) and very rarely in calves (37) and piglets (11)

The etiology of these maladies remained obscure until Bennetts and Chapman (14) showed that enzootic ataxia of lambs in Western Australia was a manifestation of copper deficiency which could be prevented by the administration of copper supplements to the ewe during pregnancy It was later shown to be associated with a low copper content of the pastures (usually 2–4 p p m) a subnormal copper status of the blood and tissues of both ewes and their affected lambs and other evidences of copper deficiency notably anemia and impaired wool growth and quality (12) Subsequent investigations have demonstrated unequivocally that the malady wherever it occurs is associated with a disturbance of copper metabolism In Australia and New Zealand where considerable tracts of copper deficient country exist it occurs as a result of a simple dietary deficiency of copper or a dietary deficiency of copper accentuated by above normal intakes of molybdenum from the pastures Elsewhere the disease is not always so clearly related to copper deficiency In the swayback areas of England for instance complete prophylaxis is achieved by supplementing the diet of the ewe with copper sulfate during pregnancy Reduced blood and liver copper concentrations in the ewe and affected lambs are of frequent occurrence but the copper content of the swayback pastures is normal or even high (7–15 p p m) and there is no strict correlation between the blood copper

level of a ewe during gestation and the appearance of swayback in her lamb All ewes bearing ataxic lambs have been found to be hypocupremic but not all hypocupremic ewes bear ataxic lambs In fact cases have been observed in which the blood copper of the ewe may fall during pregnancy to as low as 20 μg per 100 ml or less (one fifth normal) and a healthy lamb still be delivered Swayback must therefore be regarded as a "conditioned" copper deficiency—a disturbance of copper utilization by the animal brought about by the presence in the pasture of some factors or factors unknown This factor on present evidence is not molybdenum since the molybdenum content of the "affected" pastures is usually "normal"

Two types of neonatal ataxia have been recognized in lambs the *common acute form in which the lambs are affected when born and a* "delayed type" in which clinical symptoms are not shown for a few weeks or sometimes even months after birth In both the symptoms are those of a spastic paralysis which vary only in severity Incoordinated movements of the hind limbs a stiff and staggering gait and an exaggerated swaying of the hind quarters are shown as the disease develops (Fig 9) Some lambs are completely paralyzed or are ataxic at birth and soon die Under Australian conditions lambs more commonly appear normal at birth but develop the characteristic ataxic condition within a few weeks most frequently between one and two months of age This condition is progressive until locomotion for more than a few steps becomes either impossible or so difficult that in advanced cases they survive only so long as they can keep up with their mothers and secure milk or avoid fatal pulmonary infections Mild cases also occur in which the ataxia is only revealed when the lambs are startled or driven for some distance These apparently recover if their copper status is improved Appetite remains unimpaired and movements of the head and neck are not noticeably affected

Pathologically the disease is characterized by a diffuse symmetrical demyelination of the central nervous system varying in extent from small foci in the cerebral white matter to gross demyelination of both hemispheres with liquefaction and cavitation in extreme cases and always with secondary demyelination of the motor tracts of the cord These changes are irreversible and have been shown to commence as early as six weeks before birth and to continue with varying severity until delivery From the high incidence of the disease in newborn lambs or in lambs within a few days or weeks of birth it is apparent that the lesions usually originate in the fetus but this is not invariably so because

level of a ewe during gestation and the appearance of swayback in her lamb All ewes bearing ataxic lambs have been found to be hypocupremic but not all hypocupremic ewes bear ataxic lambs In fact cases have been observed in which the blood copper of the ewe may fall during pregnancy to as low as 20 μg per 100 ml or less (one fifth normal) and a healthy lamb still be delivered Swayback must therefore be regarded as a "conditioned" copper deficiency—a disturbance of copper utilization by the animal brought about by the presence in the pasture of some factors or factors unknown This factor on present evidence is not molybdenum since the molybdenum content of the "affected" pastures is usually "normal"

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ataxia very occasionally appears in young sheep twelve months or more after birth

The relation of these lesions of the central nervous system to copper



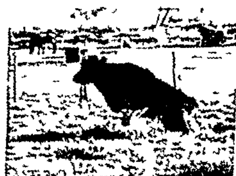





Fig 9 Enzootic ataxia in a lamb and a calf showing the incoordination of the limbs and typical swaying of the hind quarters (Photos kindly supplied by Dr I J Cunningham)

deficiency and their restriction in the spinal cord to the motor paths which serve the hind limbs as well as the particular susceptibility to copper deficiency of the nervous tissue of the fetal lamb compared with that of all other species pose intriguing questions for which there are

as yet no satisfactory answers It is known that ataxia rarely, if ever occurs unless the copper concentration in the blood of the ewe during late pregnancy falls to about 50 µg per 100 ml and more usually it falls to 20–30 µg per 100 ml but this merely indicates that the myelin cells of parts of the nervous system in the lamb cannot maintain their integrity and normal functioning when supplied with blood so low in copper It seems logical to suggest that oxidation reduction processes involving the copper protein oxidases are impaired in these cells This must remain as pure speculation however until metabolic defects involving these enzymes have been demonstrated No such changes have so far been recorded It may be also that copper is less directly involved There is some evidence that the lesions are associated with vascular and/or circulatory changes presumably as the result of functional disturbances (119) which are known to produce similar lesions in the central nervous system of human beings (74)

4 *Copper in Relation to Pigmentation of Hair and Wool*

Some degree of achromotrichia has generally been found to be associated with copper deficiency in the rat rabbit cat dog goat sheep and cattle but not so far in pigs The syndrome of achromotrichia alopecia and dermatosis according to Smith and Ellis (129) is a more sensitive index of copper deficiency in the rabbit than is anemia In the sheep there is evidence that pigmentation of the wool is the first of the many functions of copper to fail as copper deficiency develops in this species (44) Lack of pigment production in black wooled sheep and the characteristic loss of crimp from the fibers to be discussed later occur at levels of copper intake sufficient to prevent any other clinical signs of copper deficiency The pigmentation process is so quickly sensitive to changes in copper status of the animal that once a condition of *copper deficiency has been established in black wooled sheep it is possible* to produce a fleece with alternating bands of pigmented and unpigmented fibers according as copper is added to or withheld from the diet (Fig 10) Copper depletion of the animal is however not essential for the demonstration of depigmentation in black wooled sheep Even on fairly high copper intakes Dick has shown that by raising the molybdenum and inorganic sulfate intakes sufficiently it is possible to block the functioning of copper in the pigmentation process within a matter of two days He suggests that this effect may occur within hours of giving the first dose of molybdenum and sulfate Of interest in this connection was the observation that the color of the kemp or hair in black sheep (such as the hair on the nose etc) is not affected in the

same way as the wool This suggests that the mechanism of pigmentation in hair and wool may not be the same in this species

Copper containing polyphenyloxidases are known to catalyze the production of melanin from L tyrosine (110) and it seems a reasonable assumption, although there is no experimental proof of this that there



Fig 10 Wool from a black wooled sheep showing an unpigmented band grown during the period when the animal was suffering from copper deficiency (Photo by courtesy of Dr H W Bennetts)

is a breakdown in this mechanism in copper deficiency Pigmentation is however a complex process in which many nutritional factors are involved Defective pigmentation has been reported in several species as a result of various dietary deficiencies including that of p amino benzoic acid pantothenic acid pteroylglutamic acid and in the rat zinc (134) The way in which these factors operate is unknown but at least one of them namely pantothenic acid seems to be linked with copper in the pigmentation process The concentration of copper in the skin of rats which have become depigmented as a result of panto

thenic acid deficiency is about five fold that of normal animals (71), and the impaired pigmentation of rats subsisting on essentially dried milk diets can be restored to normal by an addition to the diet of either copper or pantothenic acid (127) These findings suggest the possibility that pantothenic acid is required to allow copper to function in pigment formation in the integument

5 *Copper and Keratinization*

In addition to the pigmentation defects just described marked changes in the growth and physical appearance of hair, fur, or wool have been noted in copper deficient rats rabbits dogs cattle and sheep Under conditions of copper deficiency alopecia and dermatosis develop readily in rabbits (129) the hair of dogs has been reported to become rough and dull (2) a harsh staring coat has frequently been observed in cattle (32 37) and marked changes occur in the wool of sheep It has been reported also that the plumage of Leghorn cockerels is greatly benefited by the addition of copper to their rations (141) Such evidences of copper deficiency are especially striking in the Merino sheep in which failure of the follicles to impart the characteristic crimp to the wool fibers (Fig 11) together with the failure of pigmentation in naturally dark wooled animals represent the first discernible symptoms of copper deficiency The effect on the character of the wool is less obvious in other breeds of sheep which carry wool with a much less well defined crimp

A reduction in both the quantity and quality of wool produced by sheep subsisting on copper deficient herbage was recognized early in the Australian investigations (9 101) Copper supplementation of such sheep induces an increase in the weight of wool produced the rate of wool growth being related to the degree of copper deficiency imposed (12 98) *It is doubtful however if this is a direct influence of copper* on the capacity of the follicle cells to proliferate It seems more likely that the lowered wool weights are an expression of an inadequate supply of substrate to the follicles consequent upon the somewhat reduced food consumption of the copper deficient animals This finds some support from the fact that in the experiments of Marston and Lee (98) the extent of the depression of the growth rate of the animals like that of the rate of wool growth was related to the degree of copper deficiency The deterioration in the subsequent process of keratinization however signified by the failure to impart crimp is undoubtedly a specific effect of copper deficiency

As the animals reserves of copper are used up and before the con

centration of copper in the blood has fallen sufficiently low to limit blood formation and cause anemia the crimp in the wool becomes progressively less distinct in the newly grown staple until the fibers emerge as an almost straight, hairlike growth to which the popular descriptive terms stringy or steely wool have been applied A spectacular restoration of the crimp occurs when copper supplements



Fig 11 Wool from Merino sheep The sample on the left is from a copper deficient animal and has the straight stringy or steely appearance with an absence of crimp characteristic of this deficiency The sample on the right is from a similar animal receiving adequate copper and shows the typical crimping of the fibers

are given The physical properties as well as the appearance of this wool differ markedly from those of normal wool Its tensile strength and affinity for dyes are reduced, its elastic properties are abnormal and it tends to become permanently set when stretched (96)

These drastic changes in the physical structure of the wool of copper deficient sheep are without doubt primarily the result of an impairment of the chemical mechanisms involved in the keratinization process The characteristic physical properties of wool including crimp are known to be dependent upon the presence of disulfide groups which provide the cross linkages or bonding of keratin and upon the particular align ment or orientation of the long chain keratin fibrillae in the fiber Both of

these appear to be adversely affected under conditions of copper deficiency. There is a lowered efficiency of conversion of the free thiol groups of the prekeratin fibrous protein to the disulfide groups of keratin proper. This has been beautifully demonstrated by the application of histochemical techniques capable of revealing free thiol groups. In this way Marston (95, 96) has shown that the transition zone in which reduced (–SH) prekeratin exists at the base of the wool fiber prior to its oxidation to (–S–S–) keratin is increased as much as tenfold or more in the fibers of the copper deficient sheep. It would appear that this oxidation proceeds so slowly under conditions of copper deficiency that the fiber remains plastic long enough to provide opportunity for the keratin to become disorientated before internal bonding or "fixing" by oxidation of –SH to –S–S– is complete. This process takes 3 days or more to complete in the integument of the copper deficient sheep compared with a normal period of only 8–12 hours (95). These findings indicate that adequate concentrations of copper are required in the follicle cells for the conversion of prekeratin to keratin and suggest that copper functions through the enzymic catalysis of the oxidation of the thiol groups present. Experimental proof of a reduced concentration or activity of copper containing catalysts in the integument of copper deficient sheep has, however, yet to be established.

6. *Copper and Fibrosis of the Bovine Myocardium*

In parts of southwestern Australia a disease of cattle known locally as falling disease and characterized by seasonal incidence and sudden death usually without premonitory signs was known for many years. Preliminary investigations showed it to be strictly enzootic and apparently not due to any infectious agent or toxic plant. A series of experiments begun by Bennetts and his colleagues in 1937 (15) led to the conclusion that the disease was the terminal manifestation of severe copper deficiency which could be completely prevented by copper therapy or treatment of the pastures with copper salts. The copper status of adult cows which had died of falling disease and of the affected pastures was extraordinarily low. The blood copper concentrations of such animals were only about 10 μg per 100 ml and the liver copper levels frequently as low as 2 p.p.m. on the dry basis. The copper content of the pastures on which the disease occurred usually lay between 1 and 3 p.p.m. These figures indicate the most severely copper-deficient conditions ever recorded in any part of the world.

Sudden deaths similar to those from falling disease have been reported to occur occasionally in cattle in copper-deficient areas else

where, namely, in parts of eastern Australia, New Zealand, and Florida, but they have not been a conspicuous feature of the deficiency. Moreover, the disease has never been observed in sheep or horses grazing under the same conditions, nor in pigs, rats, or dogs in which acute copper deficiency has been experimentally induced. The condition must therefore be regarded as a manifestation of very severe copper deficiency restricted to the bovine species. Affected animals, however, and other members of the herds in which the sudden deaths occur reveal other more common signs of lack of copper, namely unthriftiness, diarrhea, and anemia. A curious feature of the anemia, which may be either moderate or severe and is of the macrocytic hypochromic type, is that it exists only during the spring months of the year when most of the sudden deaths occur. It disappears spontaneously each summer, *in spite of continued very low blood copper concentrations*.

Detailed pathological studies have revealed the essential lesion to be atrophy of the myocardium with replacement fibrosis, and the sudden deaths are believed to be due to heart failure as a result of the cardiac lesions (15). The morbid process is a progressive one extending even over a period of several years, commencing with the presence of occasional areas of small-celled infiltration and proceeding to the replacement of large and widely distributed areas of atrophied myocardium with dense collagenous tissue. The extent of the fibrosis is not particularly well correlated with the occurrence of the sudden deaths, since they occur where the demonstrable lesions are relatively inconspicuous, whereas other animals affected with extensive and long-standing fibrosis have not succumbed. It is possible that the distribution of the lesions may be of more significance than their extent. Possibly also the histological picture does not give a true measure of the functional capacity of the heart. In man it is known that heart failure occurs not uncommonly in the absence of serious myocardial lesions when the subjects are severely anemic.

The exact relationship of the myocardial atrophy and fibrosis to copper deficiency has not yet been fully determined. The lesions could result from deficient oxygenation due to the anemia, or to inadequate circulation, or to both. There is no evidence for the latter, but a condition of anemia certainly operates for some months prior to death. On the other hand, equally severe anemia due to copper deficiency in ovines or other species has apparently not resulted in similar heart lesions or at least in similar sudden deaths. It seems more probable therefore that the anemia is simply a contributory factor and that bovine heart muscle is

particularly sensitive to prolonged low blood copper status It can be supposed that enzyme systems involving copper and in particular the cytochrome system which has already been implicated in copper deficiency in the rat (121), are more readily disturbed in the heart tissue of the bovine than in that of other species There is as yet no experimental evidence to support this hypothesis Cytochrome *c* and cytochrome oxidase determinations in the heart, diaphragm and skeletal muscles of a series of animals maintained for varying periods at different levels of copper intake would be of great value in throwing further light on this and other aspects of copper metabolism

7 *Copper and "Scouring" (Diarrhea) of Cattle*

Diarrhea in cattle varying in severity from a mild and transient condition to an acute persistent and debilitating scouring (see Fig 13 p 133) occurs under conditions of copper deficiency in some areas and rarely or not at all in others In Holland severe diarrhea was such a dominant symptom of an enzootic disease of cattle occurring in certain parts of the country that the name "scouring disease" was early applied to it (21 128) In New Zealand an acute scouring condition of cattle is restricted to pastures developed on reclaimed peat lands so that "peat scours" has become the popular name for the disease (39) In both cases the condition is associated with a low copper status of the blood and tissues of affected animals and can be prevented or cured by adequate copper therapy by mouth by injection or through treatment of the pastures Subnormal levels of copper in the soils and pastures of peat scours land also occur but there is no clear and consistent association of the disease in Holland with abnormally low pasture or fodder copper levels Moreover horses and sheep grazing under the same conditions do not scour as do cattle and no signs of diarrhea appear in rats pigs or dogs made severely copper deficient in the laboratory These facts suggest that the scouring does not occur as a manifestation of a simple copper deficiency and point to some complicating factor which exists in the herbage of some copper deficient areas and not in others and to which the bovine gastrointestinal tract is especially susceptible

Cunningham (37) has presented evidence implicating molybdenum as the factor concerned in peat scours and claims that the disease is in effect a molybdenosis conditioned by the low copper status of the animals Excessive ingestion of molybdenum is known to produce profuse scouring or "teart" disease in cattle which can be prevented or rapidly cured by adequate intakes of copper (58) Smaller amounts of molybdenum than those common in teart pastures have no such effect

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as long as the copper intake of the cattle is normal Typical peat scours pastures contain only a moderate excess of molybdenum but they contain insufficient copper to antagonize or nullify the pathogenic effects of this amount of molybdenum (37) On this basis the incidence of scouring in cattle is dependent upon the relative amounts of copper and molybdenum in the fodder and the condition will only occur on copper deficient pastures that contain amounts of molybdenum in excess of normal The problem of scouring in cattle will therefore be considered further in Chapter 4 where the whole question of the reciprocal antagonism between copper and molybdenum is discussed and where theories as to the ways in which these elements function in relation to gastrointestinal disturbances in the bovine are considered

It is appropriate however, to point out at this stage that varying amounts and proportions of copper and molybdenum in the herbage are of themselves, insufficient to account for the presence or absence of scouring in cattle under all field conditions Intermittent diarrhea in cattle has been reported by Bennetts and co workers (13 15) in the falling disease areas of Western Australia under conditions of severe copper deficiency but low levels of molybdenum Conversely Davis (42) found that cattle grazing certain pastures in Florida which were low in copper but had a moderate excess of molybdenum developed anemia achromotrichia anorexia and osteoporosis all of which are characteristic of copper deficiency but he makes no mention of the occurrence of diarrhea in these animals The existence of additional dietary factors influencing copper and molybdenum metabolism such as has already been postulated to account for the incidence of swayback in lambs in parts of England, appears therefore as a likely possibility The profound importance of inorganic sulfate in this connection has already been pointed out. The possible significance of high intakes of zinc also deserves mention

VI Copper Requirements

1 *Man*

The minimum copper requirements of the human species are unknown but they appear to be lower per unit of body weight or of food consumption than those of pigs sheep goats or cattle Minimum mineral requirements of animals are usually assessed from balance experiments or from the results of additions of graded increments of the element in question to known deficient diets The latter approach has not been possible in man because of the absence of naturally occurring copper

deficiency and the difficulty of achieving the deficiency state experimentally in this species Copper balance experiments in man have been carried out but they have given very variable results This is not very surprising in view of the difficulties in completely avoiding contamination with copper and the influence of variable body stores of copper in short term studies as well as the technical problems involved in the estimation of the small amounts of copper concerned

Tompsett s (137) balance experiments with adult humans indicate a minimum copper requirement as low as 0 6 mg daily The estimate of Chou and Adolph (29) is 1 to 2 mg daily with much the same requirement for children and De (43) gives a figure close to 2 mg copper daily for adults Growing children have been estimated to retain 0 06–0 10 mg copper per kg body weight (126) indicating a minimum requirement of 1 2–2 0 mg daily for an average four to six year old weighing 20 kg Wintrobe and others (143) fed diets identical with those which induced severe copper deficiency in rapidly growing pigs to two infants 5 days and 8 months of age respectively for 4 and 5 months without any sign of copper deficiency appearing This suggests that either human infants have a much lower copper requirement than weanling pigs or these particular infants commenced the experiment with exceptionally high body stores of copper

2 *Sheep and Cattle*

Considerable uncertainty now exists as to the minimum copper requirements of sheep and cattle in view of recent evidence pointing to the existence of several dietary factors which can greatly influence copper assimilation and utilization Some of these factors are as yet unknown but the inhibiting effect of molybdenum on copper absorption *and retention in these species and the vital significance of inorganic* sulfate in facilitating this inhibition indicate clearly that estimates of copper requirements based solely on copper intakes which therefore do not take into account these factors are of limited value

Clearly also there must be a series of copper requirements dependent upon the extent to which these various factors are present or absent from the diet A "true" or basic minimum copper requirement can be conceived as one in which all the dietary conditions affecting copper are at an optimum Such a requirement cannot yet be given but Dick (45) has provided evidence that in crossbred sheep at least it is much lower than has generally been believed He claims that such sheep can under appropriate conditions be in copper balance at copper intakes of

1 mg/day or less, which on normal intakes means a diet containing the dry basis, only about 1 p p m of copper (Fig 6) Calcula from Dick s data indicate further that the normal wastage of copp these animals is less than 3 mg/day and suggest that where more this amount is required by sheep to maintain their copper status, fa are operating which either impose a limitation on copper assimila and retention or increase the animal s requirement for this element

In contrast to these findings are those of Marston and his collea (97, 100), in which such limiting factors apparently operate Me sheep grazing on the highly calcareous soils of South Australia w the pastures contain about 3 p p m of copper, rapidly develop sympt of copper deficiency if supplied with additional cobalt to prevent d before signs of copper deficiency appear A supplement of 5 mg co per day (equivalent to a total pasture copper of about 8 p p m) found to be insufficient to ensure normal blood copper levels keratinization in all cases, although it was adequate to prevent o symptoms of copper deficiency From the results of this and o experiments by the same group of workers it would appear that minimum copper requirements of wool sheep under these partic conditions are close to 10 mg/day or approximately 10 p p m of diet It seems likely that these relatively high requirements are due an unknown extent, to the limiting effects of a high consumption calcium carbonate from the environment and of molybdenum and organic sulfate from the herbage on copper assimilation and utilizat

Conditions exist in Western Australia which appear to impose cop requirements between those found by Marston and implied from research of Dick Pastures in this part of the world containing 3–4 p p of copper or less and molybdenum concentrations that are usu below 1 5 p p m, induce subnormal blood and liver copper levels an whole range of copper deficiency symptoms in sheep and cattle (Pastures containing 4–6 p p m of copper on the dry basis and simil low in molybdenum provide sufficient copper for the full requireme of cattle and of British breed and crossbred sheep These types of sh are apparently more efficient in securing copper from herbage and less susceptible to changes in the wool than are Merinos If Mer sheep are to avoid all symptoms of copper deficiency including defect keratinization a minimum level of about 6 p p m of copper in su pastures appears to be necessary (6) On this basis the minimum cop requirements of cattle and British breed or crossbred sheep compati with completely satisfactory health and production can be given

about 4 p.p.m. of the moisture free diet and those of Merino sheep as about 6 p.p.m. It should be pointed out, however, that even the relatively low levels of molybdenum present in these pastures may be having some effect on the requirement since Dick (45) has shown that molybdenum intakes as low as 0.5 mg./day can adversely affect copper retention in sheep provided the sulfate intake is high.

These findings and others cited earlier in the sections dealing with the conditions under which the various manifestations of copper deficiency occur emphasize the complexity of the problem of copper requirements in these species and point to the necessity for much further experimentation under closely controlled pen feeding conditions to enable this problem to be fully elucidated.

3. *Other Mammals*

From the limited evidence available it appears that the minimum copper requirements of goats and foals are very similar to those of sheep and cattle but the requirements of adult horses are appreciably lower. Complete freedom from any evidence of copper deficiency has been reported in adult horses in all areas where copper deficiency occurs in sheep and cattle, including those in which falling disease of cattle is enzootic (15), but foals do not thrive and they develop the bone abnormalities previously mentioned when grazed under these conditions (9, 10). Since the pastures of these latter areas usually contain only 1–2 p.p.m. of copper the minimum copper requirements of adult horses can be placed at not more than 1–2 p.p.m. of the ration. They may, of course, be considerably less.

The minimum requirements of rats and pigs have not been established very clearly in spite of the large amount of work with these species and the relative ease of inducing copper deficiency with milk diets. In the rat supplements of copper as low as 0.005 mg. per rat per day give a distinct response on such diets but the optimum level is stated to be between 0.01 and 0.05 mg./day (125). It was reported later (125) that a supplement of 2–4 mg. copper per pig per day is sufficient for hemoglobin formation during the recovery of young pigs from anemia produced on a whole milk diet supplemented with iron and manganese, but it is not claimed that this is the minimum level compatible with satisfactory growth and blood formation under these conditions. From the experiments of Teague and Carpenter (136) it appears that an over all intake of 0.035 mg. copper per lb. body weight is insufficient for young growing pigs and that an intake of 0.05–0.07 mg. per lb. body weight is close to the minimum.

The minimum copper requirements of poultry have never been accurately determined either for growth in chicks or for egg production in laying hens despite the fact that this element within a year of its original demonstration as an essential *element in the nutrition of rats* was shown to be necessary for growth and hemoglobin formation in chicks (53) This no doubt is due to the absence of any practical problem of copper deficiency in this species such as occurs in the case of manganese It is clear however, that the copper requirements of birds *in contrast to their relatively high requirements* of manganese, are of the same order as those of mammals Any practical chick or laying ration supplies ample copper for the needs of birds and very special diets are necessary for the demonstration of copper deficiency

The demand of the laying hen for copper is not inconsiderable since the average egg contains *0 03–0 06* mg of this *element* but a reduced concentration in the egg sufficient to affect hatchability or the development of the chick embryo has not been reported Considerable variability in the copper content of eggs occurs for reasons not yet fully understood Feeding copper supplements to hens receiving normal diets is ineffective in raising the amount of copper in the egg (54) This is similar to the position with iron but contrasts greatly with the position *with iodine and manganese and several other trace metals where as* is shown later very substantial increases in the concentrations in the egg can be achieved by such means

VII Copper in the Nutrition of Man

The value of dietary supplements of copper, with or without iron in the treatment of anemias of infancy and of secondary anemias of adults has been extensively studied with very varied results Iron alone has frequently been found to be just as effective as iron and copper A few cases have been reported in which copper therapy has been successful when iron therapy has failed and a number of workers have found that iron and copper together give results in the treatment of nutritional anemia of infants superior to those of iron alone Hutchinson (72) reviewed most of the literature on this subject up until 1938 and presented some evidence of his own which appeared to demonstrate the value of copper in addition to iron *Perhaps the most striking demonstration* of the value of copper is that of Usher and co workers (139) who found 1–2 mg copper per day, plus iron to be markedly superior to iron alone in producing and maintaining hemoglobin levels in a prophylactic

experiment with a large group of infants Many cases of chronic idiopathic anemia of adults refractory to iron have also been reported to respond to copper therapy (107) The claim that copper therapy is useful in the treatment of human anemias has not been free from criticism some of it exceedingly trenchant Wintrobe and co workers (143) in fact categorically deny that true copper deficiency has ever been recorded in man They studied 24 cases of hypochromic microcytic anemia in infants and in a number of repatriated prisoners of war who had suffered from starvation and in whom hypoferremia was present without being able to demonstrate hypocupremia in any one of them These workers were also unsuccessful in inducing any sign of copper deficiency in two infants fed diets identical with those which were highly successful in producing such a deficiency in young swine

Such findings conflicting though they are make it obvious that copper deficiency in man can occur but that it must be exceedingly rare at any age They reveal further that this element is of minor importance in human nutrition compared with iron Nevertheless the number of cases of human nutritional anemia in which copper therapy has been found necessary for maximum response is sufficiently large to warrant the inclusion of some copper in iron prescriptions at least for the anemias of infancy and childhood

Various estimates have been made of the average daily intakes of copper from ordinary diets These vary over quite a wide range but a typical adult diet reasonably satisfactory in other respects generally supplies between 2 and 3 mg copper per head per day (83 137) Slightly lower intakes were found for certain English and Scottish families consuming relatively poor quality diets (40) and substantially higher intakes of 4 5 mg and of 5 8 mg for Indian adult males consuming rice and wheat diets respectively (43) The magnitude of the daily intake depends upon a number of factors of which the proportion of particular copper rich or copper poor classes of foods in the diet is normally the most important Intakes of copper may also be influenced by locality in view of the relation between the copper content of many foods and the soil and climatic conditions under which they are grown Adventitious sources of copper from contact of food and drinking water with copper pipes processing vessels and cooking utensils and from treatment with copper fungicides can increase intakes significantly

Excluding the influence of adventitious additions of copper which are becoming less important under modern conditions and as public health regulations are made more stringent and the considerable varia

bility in the copper content of particular foods due to varietal and environmental influences, the various classes of foods which comprise the main items in most modern dietaries differ significantly and fairly consistently in average copper content The results of determinations of the copper contents of the edible portions of a wide variety of American (86) and English (105) foods have been compiled and very many others have been published It is apparent from these analyses that the richest sources of copper are the organ meats (liver, kidney, heart and brain) crustaceans and shell fish (especially oysters), nuts, dried legumes, dried vine and stone fruits and cocoa The copper content of these foods ranges from about 20–30 p p m to as high as 400 p p m The poorest sources of copper are milk, butter cheese white sugar honey, lard, and margarine, which rarely contain more than 0 5 p p m closely followed by the nonleafy vegetables most fresh fruits and the refined cereal products including white flour and bread, polished rice and corn flour These materials have copper contents up to about 2 p p m Between these two groups lies a miscellaneous group of foods of intermediate copper content This includes wholemeal flour and bread and other whole cereals, the green leafy vegetables, eggs muscle meats, fish and poultry The similarity between this classification of foods as sources of copper and that made earlier for foods as sources of iron should be noted With the exception of the crustacea and shellfish which are exceptionally rich in copper those classes of foods which are high in iron are also high in copper and those which are low in iron are also generally low in copper

The influence of locality on copper intakes from human dietaries is of particular interest in relation to those areas where copper deficiency in grazing stock is enzootic The question has frequently been asked if human populations living in these areas may not also suffer from subnormal intakes of copper Since copper deficiency in animals, expressed in the simplest terms, results from subnormal concentrations of copper in the herbage in consequence of subnormal total or available concentrations of copper in the s[illegible] which the herbage grows it
would be expected that, on [illegible] plants grow[illegible] for
human consumption such as [illegible] fruits and [illegible]
similarly be below normal in [illegible] at There [illegible] at
this is usually so The nature of [illegible] of plants to
soils depends primarily upon [illegible] the degre[illegible]
Invari[illegible]r, there is a [illegible]
in th[illegible] There is [illegible] with

at all and the development of certain specific symptoms of deficiency This question is considered further in Chapter 13 Seeds fruits and tubers however appear to be less sensitive in their copper contents to changes in the copper status of the soil than are the vegetative parts of the plant so that cereal grains and fruits and nonleafy vegetables would not normally be reduced in copper content to the same extent as would the leafy vegetables when both are grown on deficient soils In one study (61) wheat barley, and oats were each grown on different soils ranging in copper content from 4 p p m, which can be regarded as mildly copper deficient to the exceptionally high figure of 51 p p m The copper in the grain varied from 5 6 to 16 7 p p m but bore no significant relation to the copper content of the soil

It seems therefore that any community living in a copper deficient area which is solely or largely dependent for its food supply on the products of that area is likely to have a lower daily intake of copper than a community with similar food habits living in another locality where the soils are higher in copper status It must be remembered that not only would the foods of plant origin tend to be lower in copper content in the deficient area but also the foods of animal origin including particularly such organ meats as liver and kidney which are among the richest sources of copper known It cannot be assumed however that such a diet would supply insufficient copper for human requirements Certainly no convincing evidence of malnutrition or malaise in humans specifically attributable to lack of copper in a particular area has been presented so far as is known Moreover modern technical developments make it very unlikely that any such condition will arise in the future The first of these developments is the relative ease with which copper deficiency in both plants and animals can now be recognized and overcome by means of treatment with copper salts which not only raises productivity and eliminates symptoms of disease but increases at the same time the concentrations of copper in the plant and animal tissues and hence in the human foods The second important modern development is the decreasing dependence of communities in particular areas upon the food products of that area due to the ever widening source of supply The food of man comes not from one but from a multiplicity of soil types This question was discussed in Chapter 2 (iron) and is considered again in Chapter 9 (iodine) and in Chapter 13 (soil plant animal *interrelationships*)

VIII Copper Toxicity

1 *General*

The toxic nature of copper salts in large doses has been known for centuries but the serious effects in some species, of relatively small doses over long periods has only become recognized in recent years The possibilities of acute and chronic copper poisoning in animals assumes particular importance in the light of the increasing use of copper compounds in agricultural and veterinary practice and in the prophylactic treatment of the various copper deficiency diseases of sheep and cattle in certain areas Chronic copper poisoning in sheep to be discussed later, occurs also under natural conditions in Australia (1, 25) It is in fact, largely through studies of this disease and means of preventing it, that the recent substantial advances in our understanding of the factors influencing copper assimilation and retention have been accomplished (44–46)

Continued ingestion of copper in excess of the nutritional requirement leads in all animals, to its passive accumulation within the tissues especially the liver which has a remarkable ability to take up and retain large amounts of ingested copper Up to certain levels, which vary greatly with the species and the individual the very high concentrations of copper in the liver appear normally to impose no physiological hardship on the animal Above these levels for reasons not fully understood there may occur a catastrophic liberation of a high proportion of the copper into the blood stream with resultant extensive hemolysis and jaundice followed usually by death British breeds of sheep and their crosses are more prone to the hemolytic crisis of chronic copper poisoning than Merinos (1 97) but sheep generally accumulate copper more readily than do other species including cattle under similar conditions of high copper intake (38) Hemolytic jaundice however has been reported in cattle that have been dosed for a period with large amounts of copper (76) Accumulations of copper occur in rats and rabbits when subjected to chronic overdosage with this element but hemolytic jaundice does not appear to be a feature of chronic copper poisoning in these species (19 64)

In rats copper does not begin to exert toxic effects until at least 100 times the normal intake from ordinary diets has been reached judging by the results of experiments with this species lasting only 4 weeks (19) In these experiments rats maintained good growth and food consumption and appeared normal in every respect on diets containing 500 p p m of copper equivalent to an intake of about 5 mg /day in spite

of significant increases in blood and spleen copper concentrations and a 14 fold increase in liver copper At higher intakes of copper there is invariably a marked depression of growth and food consumption with rapid loss of weight and death in some animals Bovines have also been shown to exhibit a high resistance to repeated doses of copper sulfate No ill effects have been observed from feeding adult cows 1 2–2 g copper sulfate daily for 5 to 18 weeks (56) or from feeding 0 8–5 g daily for 9 months or longer to growing calves and adult cows (38) These intakes are from 4 to 10 times the normal and indicate the considerable margin of safety which exists between prophylactic or therapeutic levels of copper and those likely to produce toxic effects in this species

2 *Chronic Copper Poisoning in Sheep*

In the sheep the position is quite different Heavy losses of sheep from chronic copper poisoning with hemolytic icterus and hemoglobinuria as characteristic clinical signs have been reported from various parts of the world as a result of ingestion of excessive amounts of copper from the foliage in vineyards and orchards sprayed with copper fungicides (17) or from salt licks containing copper sulfate for the control of parasitic worms (17) Occasional outbreaks have occurred also in copper deficient areas through chance overdosage with copper These may however all be classed as accidental or artificially induced occurrences Of much greater interest is the occurrence of chronic copper poisoning in sheep under natural grazing conditions in parts of eastern Australia (1 25) There appears to be no malnutrition associated with the condition and Bull (25) has characterized the disease in the following terms " a more or less sudden hemoglobinemia and hemoglobinuria usually associated with the occurrence of icterus which increases rapidly The hemolytic crisis is associated with necrosis of the liver kidney dysfunction and so called uremia The copper concentration in the liver is usually higher than 1000 p p m on a dry fat free basis The presence of hemoglobinuria in the sheep is highly presumptive of chronic copper poisoning If this is associated with central necrosis of the liver lobules with a premonitory rise in blood copper which may be ten fold in magnitude and with a high copper content of the liver then a diagnosis of chronic copper poisoning can be made The critical factor in the precipitation of the hemolytic crisis has been postulated as a high concentration of mobile or active copper (25) Copper becomes toxic to the cell when it is liberated in sufficient concentration from its combination with organic molecules within the cell The toxic con

copper poisoning Subsequent investigations have not supported this claim and there is no real evidence that ingestion of copper within reasonable limits can produce any such condition in man

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CHAPTER 1

MOLYBDENUM

I Historical Background

Recent evidence suggests that molybdenum might now be added to the growing list of trace minerals essential in the nutrition of the higher forms of animal life (42 44) Some years earlier this element had been demonstrated in minute amounts in a wide range of plant and animal tissues (47) but as with so many of the trace elements no special significance was at first attached to its presence The first evidence of a biological role for molybdenum came in 1930 when experiments with the nitrogen fixing soil organism *Azotobacter* showed it to be an essential constituent of the media used for the growth of this organism (10) It was later shown that molybdenum is required by all nitrogen fixing organisms, including the legume symbiont *Rhizobium sp* as well as by *Aspergillus niger* (45) and by the higher plants (2) In certain areas some plants notably the Brassicas and tomatoes have been shown to exhibit molybdenum deficiency symptoms as a direct result of lack of available molybdenum in the soils and to respond remarkably to treatment of the soil with small dressings of molybdate In other areas equally striking responses to molybdenum have been demonstrated with legumes but judging from the effects of application of nitrate it appears that these responses are not due to a direct effect of the soil deficiency of molybdenum on the plants themselves but are secondary to an increased nitrogen supply through the action of the molybdenum on the nitrogen fixing rhizobia (1)

In animals attention was first directed to the metabolic significance of molybdenum by its association with a disease of grazing cattle known as "teart" occurring on certain soil types in several of the counties of England The drastic diarrhea or "scouring which characterized this disease was shown in 1938 to be due to the ingestion by the cattle of excessive amounts of molybdenum from the herbage of the affected areas (27) The resemblance of the symptoms of teart to those exhibited by cattle in certain copper deficient areas in Holland (12) led to the successful use of copper in the treatment of this disease (27) The use of molybdenum in the treatment of chronic copper poisoning of sheep in Australia arose independently as a result of earlier observations of

unusually high concentrations of molybdenum in certain tissues of cattle suffering from enzootic hematuria vesicalis or "red water" (22). From these latter investigations and those of "peat scours" of cattle in New Zealand, came a realization of the profound effect of molybdenum on copper metabolism in the ruminant and of the dependence of this effect upon the inorganic sulfate content of the diet. Certain of these findings have already been considered in relation to copper deficiencies and excesses in grazing sheep and cattle. They are discussed further in the appropriate sections of this chapter.

Interest in the biological significance of molybdenum received a further stimulus from recent demonstrations that this element is an integral part of the prosthetic group of the two flavoprotein enzymes, xanthine oxidase (42, 44) and nitrate reductase (38). The discovery that xanthine oxidase is a molybdoflavoprotein arose from studies of dietary factors influencing the concentration of this enzyme in the liver of rats. Liver residues, soy flour, and certain other natural materials were found to increase the tissue level of xanthine oxidase in rats fed synthetic diets. Fractionation of these materials led to the identification of the "xanthine oxidase factor" as molybdenum (42, 44). This was the first indication of an essential role for molybdenum in animals.

The discovery that nitrate reductase is a molybdoflavoprotein arose from studies with *Neurospora* and *Aspergillus*. Cell-free extracts of these organisms grown in molybdenum-deficient media revealed a striking decrease in nitrate reductase activity which could be increased by graded applications of molybdenum to the nutrient medium (39). Examination of the nitrate reductase resulted in the identification of molybdenum as the metal constituent of the enzyme, whose specific activity increased with its molybdenum content. This finding links up with an earlier observation that the concentration of protein and of soluble nitrogenous constituents falls markedly, and that of nitrate rises markedly, in the tissues of molybdenum-deficient plants (37). Whether molybdenum serves any functions in plants and microorganisms other than in the reduction of nitrate is not definitely known, although there is already an indication of this from the finding that *Neurospora* and *Aspergillus* need this element in their growth media when nitrite or ammonia, as well as nitrate, represents the source of nitrogen.

II Molybdenum in Animal Tissues and Fluids

1 *Distribution Throughout the Body*

Vertebrate tissues are normally very low in molybdenum From the relatively few values available the normal concentrations appear to be similar to those of manganese and very much lower than those of copper There is some evidence that invertebrate tissues on the other hand contain appreciably higher levels of molybdenum (8) Mammalian muscle tissue usually contains 0 05–0 10 p p m molybdenum on the dry basis with similar or slightly higher concentrations in other tissues with the exception of the liver where values between 1 and 3 p p m are common

The concentrations may be raised to very much higher levels especially in the bones liver and skin by oral administration of molybdenum the values being dependent on the level of intake and the nature of the molybdenum compound fed (16 26) and in the sheep on the inorganic sulfate intake (17) Thus Davis (16) found the molybdenum content of the bones of rats to increase from 0 2 p p m to 9–12 p p m and of the livers to increase from 1–2 p p m to an average of 11–12 p p m on the dry basis when the molybdenum content of their diet was raised from below 1 p p m to 30 p p m Very much higher concentrations than these (over 100 p p m of fresh tissue) were found in the bones liver kidney and spleen of guinea pigs fed highly toxic or lethal doses of calcium molybdate or of molybdenum trioxide (26)

That the effect of high molybdenum intakes on the levels of molybdenum in the tissues of sheep is dependent upon the level of dietary intake of inorganic sulfate has been demonstrated by Dick (17) In Table 16 are shown the amounts of molybdenum in the various tissues and the total molybdenum in the bodies of crossbred sheep at two levels of molybdenum and two levels of sulfate intake From the figures in this table it is seen that the amounts of molybdenum present in the tissues are smaller when the sulfate intake is higher both at the low and the high molybdenum intake levels This is due to the marked influence of sulfate on urinary molybdenum excretion to be described later Calculations made by this worker showed that from one half to three quarters of the total molybdenum in the body is situated in the skeleton The next largest amounts in these animals occurred in the skin wool and muscles

2 *Molybdenum in the Liver*

The figures cited for the rat guinea pig and sheep indicate that molyb denum does not readily accumulate in excessively high concentrations in the liver (or elsewhere in the body) as can occur with iron and copper at high intakes of these elements This is believed to be due to

TABLE 16
THE INFLUENCE OF DIETARY MOLYBDENUM AND INORGANIC SULFATE INTAKE ON THE MOLYBDENUM CONTENT OF THE TISSUES OF SHEEP (17)

	Molybdenum content (mg)			
	0 3mg /day Mo intake		20 8mg /day Mo intake	
	0 9g /day sulfate intake	6 3g /day sulfate intake	0 9g /day sulfate intake	6 3g /day sulfate intake
Liver	1 58	0 48	5 79	1 93
Kidney	0 17	0 02	1 17	1 32
Spleen	0 52	0 02	0 57	0 14
Heart	0 18	0 01	0 94	0 04
Lung	0 65	0 09	3 96	0 42
Muscle	5 84	0 08	28 6	1 92
Brain	0 01	0 01	0 09	0 01
Skin	6 62	1 50	58 9	3 44
Wool	15 2	0 99	26 9	1 14
Small intestine	0 26	0 03	0 88	0 79
Cecum	0 24	0 01	1 64	0 58
Colon	0 63	0 04	4 21	0 62
Skeleton	61 0	13 0	164 0	16 0
Total body Mo (mg)	92 9	16 8	297 7	28 4

the relatively rapid and efficient excretion of molybdenum A number of studies has revealed the transient nature of molybdenum storage in the tissues and the rapidity with which the concentrations return to normal levels following the cessation of molybdenum administration

In sheep and cattle on low molybdenum diets the average concentra tion of molybdenum in the liver is of the same order as that found for rats namely 2–4 p p m (15 22) Similar molybdenum levels occur in the liver of newborn lambs, indicating that there is normally no storage of this element in the fetal liver during pregnancy Ready placental transfer can occur however because concentrations 3 to 10 times these normal levels have been found in the livers of newborn lambs from ewes receiving a high molybdenum diet (15) Adult sheep and cows may also retain in their livers molybdenum levels of this order (25–30

p p m) as long as they are being supplied with high or moderately high intakes of this element but the extent of the retention is only partly dependent upon the level of molybdenum intake It is highly dependent also upon the amounts and the proportions relative to the molybdenum of the copper and the inorganic sulfate intakes Under any known conditions however liver molybdenum concentrations are not excessive and it is apparent that this organ has a very limited capacity for molybdenum storage Even where the diet was high in molybdenum and low in sulfate only about 2% of the total body molybdenum of sheep was found in the liver (17)

The limiting effect of copper on molybdenum storage is shown by the findings of Cunningham (15) This worker drenched one group of young cattle grazing low copper low molybdenum herbage with sodium molybdate at the rate of 150 mg Mo 3 times a week and a second group on the same grazing with the same amount of molybdenum plus 100 mg Cu 3 times a week After some months the former group averaged 26 p p m of molybdenum (dry basis) in their livers compared with only 7 p p m in the group receiving both copper and molybdenum and 5 p p m in untreated controls The sulfate intakes of these animals are not known

Direct evidence of the effect of different intakes of inorganic sulfate on liver molybdenum levels has been obtained by Dick (17) in his studies of the profound influence of this dietary constituent on molybdenum excretion and retention in the sheep The results of one experiment are illustrated in Table 16 In another study this worker found the mean liver molybdenum concentrations of 5 sheep on a lucerne chaff diet supplying 10 3 mg Mo per day and 4 4 g inorganic sulfate per day to be 5 7 p p m whereas the mean liver molybdenum of 5 similar sheep on an oaten chaff diet supplying the same amount of molybdenum but only 0 2 g inorganic sulfate per day was 22 4 p p m In further experiments to be described later a single dose of sulfate was found to reduce the amount of molybdenum already stored as a result of its potent effect on urinary excretion of this element

3 *Molybdenum in Blood*

Very few values for the molybdenum content of blood exist other than for sheep and cattle In these species the concentration is exceedingly low when the diet is low in molybdenum and high in sulfate but can rise to very high levels under high molybdenum low sulfate conditions Beck (7) found sheep grazing green pastures normal in copper and low in molybdenum to average 1 μg Mo per 100 ml of blood

Under dry feed conditions where the diet contained a proportion of low-sulfate cereal hay the molybdenum level in the blood of these sheep rose to a mean of 6 μg per 100 ml. Cunningham (15) gives a value of 6 μg Mo per 100 ml for both sheep and cattle grazing pastures normal in copper and low in molybdenum content. Molybdate drenches given to such animals, equivalent to a dietary intake of 30 p.p.m. of molybdenum, raised the level to 60–80 μg per 100 ml in young cattle and to 240–340 μg per 100 ml in breeding ewes. In England daily doses of molybdate or dressings of the pasture sufficient to raise its molybdenum content to 40 p.p.m. increased the blood molybdenum levels of sheep and cattle to as high as 200 times the levels found in untreated animals grazing pastures normal in molybdenum content (30).

The experiments of Dick (19, 20) with pen-fed sheep given a range of diets supplemented with varying amounts of molybdenum revealed striking differences in blood molybdenum levels which were highly correlated with differences in the inorganic sulfate intakes. Thus sheep fed chaffed lucerne hay high in sulfate and supplemented with copper and molybdenum to give over-all intakes of approximately 10 mg./day of each of these elements averaged 16 μg Mo per 100 ml of blood, whereas similar sheep receiving chaffed oaten hay low in sulfate but similarly supplemented averaged no less than 675 μg Mo per 100 ml. The effect of sulfate, which was confirmed by tests with pure salts, is not confined to high molybdenum diets, although it is most marked under these conditions, since even when the molybdenum intake was only 0.3–0.5 mg./day the concentration in the blood of the sheep on the low sulfate hay was much higher than it was in the sheep on the high sulfate hay. The actual values recorded in these experiments are given in Table 17.

TABLE 17
BLOOD MOLYBDENUM LEVELS OF SHEEP[a] (19)

Diet	Intake (mg./day)		Blood molybdenum
	Copper	Molybdenum	(μg per 100 ml.)
Chaffed lucerne hay (high sulfate)	7.0	0.5	1.7
	9.8	0.5	0.4
	9.8	10.5	16.2
Chaffed oaten hay (low sulfate)	2.5	0.3	11.3
	10.0	0.3	18.0
	10.0	10.3	675.0

[a] Mean values of groups of 5.

Astonishingly high values for the blood of rabbits following a single drench of 100 mg of molybdenum trioxide (MoO_3) have been recorded (26) Within 5 6 hours of the administration of this dose the concentration of molybdenum had risen from a pretreatment level of 130–200 μg per 100 ml to as high as 1850–1970 μg per 100 ml No information is given as to the nature of the basal diet, so that it is not known whether the above pre treatment values can be considered normal" for this species

4 *Molybdenum in Milk*

Data on the molybdenum content of milk are so few that the effects of stage of lactation and of variations in intakes of molybdenum or other dietary factors on the lactating animal are unknown There is a suggestion however of an interesting and unusual species difference Drea (24) detected this element in cow s milk by a spectrographic method but was unable to do so in goat s milk This observation led to a chemical investigation of a small series of samples of milk from these two species (46) Cow s milk was found to average 47 5 μg Mo per liter (range 40–56) and goat s milk 13 5 μg /l (range 11–16) It would be of some interest to re-examine the position under a range of dietary conditions

The discovery that xanthine oxidase is a molybdoflavoprotein is likely to stimulate renewed interest in the molybdenum of milk because milk is a relatively rich source of this enzyme Evidence that molybdenum is a component of the xanthine oxidase molecule was in fact obtained by injecting sodium molybdate labeled with Mo^{99} into a cow and isolating the xanthine oxidase in the milk collected one to several days later (49) Highly purified preparations of xanthine oxidase can be obtained fairly readily from milk (6) but the proportion of the total molybdenum normally present in this form is unknown

III Absorption and Excretion

In contrast to iron and copper molybdenum is readily and apparently rapidly absorbed from the intestinal tract This applies especially to hexavalent water soluble forms of molybdenum such as sodium or ammonium molybdate which have been used in most experimental studies and to the molybdenum of herbage a high proportion of which in high molybdenum pastures at least is water soluble With rabbits and guinea pigs there is evidence that even such insoluble compounds as molybdenum trioxide and calcium molybdate are also readily and rapidly absorbed when these compounds are fed in large doses but this is not true for molybdenite (MoS_2) fed similarly (26) It is not yet known whether

molybdenum is absorbed throughout all regions of the gastrointestinal tract or whether, as with iron and copper there is a particular absorptive region Little is known, also of the factors which influence molybdenum absorption The limiting effect of copper and of inorganic sulfate on molybdenum retention is due not to reduced absorption, but to increased excretion of this element Sulfate as will be shown is extremely potent in this respect in the sheep

Most of the available evidence indicates that molybdenum is excreted mainly in the urine Studies with guinea pigs and rabbits administered various forms of stable molybdenum over a wide range of doses support this statement Support is also given by an investigation in which radioactive Mo^{99}, as labeled sodium molybdate, was administered to a steer When given by mouth about 34% of this molybdenum appeared in the feces during 14 days and 45% in the urine Following intravenous injection about 11% of the dose appeared in the feces and 37% in the urine after 6 days (13) From studies with sheep it is apparent that the total amount of molybdenum which appears in the urine is markedly influenced by the inorganic sulfate intake of the animal (21) So powerful is this factor that investigations of molybdenum retention and excretion in this species and probably also in the bovine are largely valueless unless the sulfate status of the diet is taken into account

The effect of sulfate is illustrated by the following facts taken from Dick s investigations In one experiment in which sheep were fed a diet of oaten chaff (< 0 03% sulfate) plus 10 mg molybdenum per day 63 3% was recovered in the total excreta during a period of 4 weeks of which only 3–4 6% appeared in the urine When such sheep were fed a diet of lucerne chaff (0 3% sulfate) plus the same amount of molybdenum the recovery in the total excreta was 96 4%, of which 50–53 9% appeared in the urine The actual outputs in the feces were very similar on each diet (21) Subsequently the diet of such animals was twice changed from the low sulfate oaten chaff plus molybdenum diet to the high sulfate lucerne chaff plus molybdenum diet The blood molybdenum concentrations fell markedly following each change and the output in the urine rose from 0 3 to 15 9 mg /day on the first change and from 0 3 to 13 2 mg on the second change to the high sulfate diet The daily output of molybdenum in the feces by contrast rose only from 6 9 to 9 1 mg and from 5 0 to 7 9 mg On each occasion the rise in urinary molybdenum was accompanied by an increase in urinary volume but as is shown in the next paragraph the increased urinary excretion of molybdenum is not a passive result of the greater volume of urine

Proof that these striking differences in both the extent and the route of molybdenum excretion are due to differences in the inorganic sulfate contents of the diets and not to other dietary differences was elegantly shown by fractionation of extracts of the lucerne chaff and by supplementing the oaten chaff diet with pure salts (21) A single oral dose of 11 g of potassium sulfate induced an immediate fall in blood molybdenum accompanied by a rapid rise in urinary molybdenum excretion (Fig 12) The total amount of molybdenum lost by the sheep within

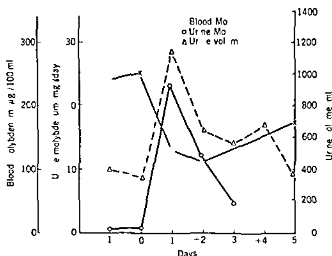


FIG 12 The effect on blood and urine molybdenum of 11 g potassium sulfate given by mouth at the time indicated by the arrow to a sheep on a diet of chaffed oaten hay and a molybdenum intake of 10 mg /day (Dick 21)

18 hours of such a dose was actually greater than could be accounted for by the fall in blood molybdenum The effect of the raised sulfate intake on excretion was evidently sufficient to induce a loss of stored molybdenum from the tissues Sodium sulfate produced similar effects *without any diuresis* whereas potassium chloride had no effect on urinary excretion of molybdenum but produced a rise in urinary volume similar to that obtained with potassium sulfate

These important findings do not necessarily apply to all mammals Evidence on this point must await further experimentation with other species It is obvious however that in the sheep molybdenum is poorly excreted mostly in the feces on low sulfate diets and that it is readily excreted mostly in the urine on high sulfate diets The quantitative relationships between the two elements have not yet been worked out nor has the mechanism of action of sulfate in promoting urinary excre

tion of molybdenum yet been elucidated The action of sulfate in facilitating the limiting effect of molybdenum on copper retention previously discussed, is equally obscure These effects may be manifestations of the same basic mechanism but clear evidence for this has not, so far, been obtained

IV Molybdenum Toxicity

The toxic effects of the trace elements concern us only in so far as they relate to an understanding of their nutritional physiology Studies of the effects of excessive ingestion of molybdenum under both naturally occurring and experimental conditions have been particularly fruitful in this respect and have led also to a very much better understanding of the metabolism of copper and of the conditions under which copper deficiency or excess in animals can occur It seems advisable therefore to consider the conditions associated with molybdenum toxicity prior to any further discussion of copper molybdenum interrelationships

1 *Ruminants*

Certain pastures in very restricted areas in England have been known for over 100 years to cause the disease teart which is characterized by severe scouring (diarrhea) and loss of condition in cattle confined to these pastures Similar effects have been reported in cattle confined to particular grazings in California (11) and in New Zealand where the disease which was described in Chapter 3 is known as peat scours All cattle are susceptible to teart but milking cows and young stock suffer most Sheep are also affected but very much less obviously and horses do not suffer from the disease at all In cattle the scouring varies from a mild form in some areas to such a severe and debilitating condition that the animals may eventually suffer permanent injury or death Within a few days or even 24 hours of being turned on to some teart pastures cattle will begin to scour profusely lose condition rapidly, and develop harsh staring discolored coats (Fig 13) They usually recover rapidly on transfer to non teart pastures

Preliminary investigation of the problem enabled the exclusion of various possible causes including infection parasites and poisonous plants and suggested that the malady resulted from either a deficiency or an excess of some mineral element in the affected herbage (36) Spectrographic examination of soils and herbage disclosed one striking fact—the molybdenum contents of the teart samples were many times higher than those of the non teart samples (27) More thorough in

vestigation by chemical means demonstrated that the teart soils contained 0 002–0 010% molybdenum in the surface horizon and that they were neutral or alkaline in reaction and often calcareous It was shown also that the teart pastures contained 20–100 p p m of molybdenum in the dry matter compared with some 3–5 p p m in nearby healthy pastures

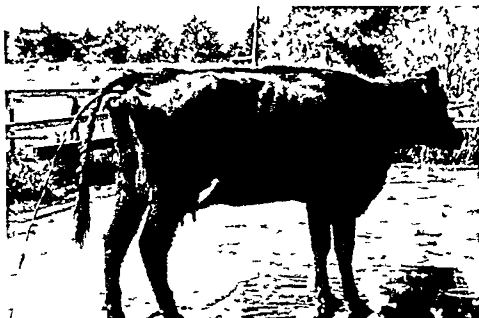


FIG 13 Cow suffering from "peat scours (Photo by courtesy of Dr I J Cunningham)

(33) These highly suggestive findings were supported by tests with added molybdenum either fed direct to the cattle as sodium or ammonium molybdate or applied to healthy pastures to bring their molybdenum content up to the levels of teart herbage (27 30) These treatments were highly successful in rapidly inducing the typical scouring of naturally occurring teart and in raising the molybdenum concentrations of the blood and tissues of the treated cattle to the very high levels characteristic of the natural condition (30) Teart was thus unequivocally established as a straightforward molybdenosis consequent upon excessive ingestion of molybdenum from the affected herbage

Attempts to mitigate the severity of the malady by cultural procedures had begun when a report from Brouwer and co workers in Holland appeared in which a rather similar type of scouring occurred which was associated with a copper deficiency and responded to relatively massive

copper therapy (12). Subsequent evidence has disclosed that the scouring disease in Holland is not caused by high intakes of molybdenum and that teart is not associated with subnormal intakes of copper. Treatment of teart with copper sulfate was nevertheless tried and found to be highly effective. Either feeding copper sulfate at the rate of 2 g/day to cows and 1 g/day to young stock, or intravenous injection of very much smaller quantities, quickly controlled the scouring in cattle whether they were grazing teart pastures or were fed ordinary diets with molybdenum added in otherwise toxic amounts.

The mechanism of the scouring effect of molybdenum and its control by copper is not yet understood. It apparently concerns only the alimentary tract but there is no appreciable inflammation or local damage of the intestines even in badly affected animals. In peat scours the onset of scouring is delayed until the normal stores of copper in the liver have been depleted and is quickly overcome by supplying additional copper either parenterally or *per os*. In teart the scouring is equally quickly prevented by copper administered in these ways but its onset or maintenance is not necessarily associated with a lowered copper status in the animal nor its treatment with copper with any marked or significant depression of the molybdenum concentrations in the blood and tissues (30). *It is apparent therefore, that copper does not act simply by* reducing molybdenum absorption through the formation of some insoluble copper molybdenum complex in the bowel. In any case this would seem to be unlikely in view of the remarkable effectiveness of relatively small amounts of *injected* copper in controlling the scouring although it has been suggested that such injected copper could reach the lumen of the intestine. It seems therefore, that the scouring arises from an obscure local effect of the molybdenum on the intestine and that copper exerts its effect in some equally obscure manner at this site. Molybdenum copper interrelations are considered more fully below.

Dick (17) has drawn attention to the possible significance of the sulfate radicle in the treatment of teart with copper sulfate. He points out that although drenching with a number of metals other than copper was shown by Ferguson and co workers (27) to have no effect on the scouring the chloride was used in every instance. Copper alone was given as the sulfate. In a preliminary experiment Dick (17) was able to show that molybdenum induced scouring could be controlled in a single calf within 4 days by daily drenching with 2 g copper sulfate and within 6 days by daily drenching with an equivalent amount of sulfate as potassium sulfate. These interesting observations need confirm

ing and extending but they suggest that the effect of copper sulfate in overcoming molybdenum scours in cattle may be due at least in part to the sulfate part of the molecule

At very high intakes of molybdenum such as occur on typical teart pastures the severity of the scouring and loss of weight obscures other effects of this element In some areas moderately high molybdenum intakes have been shown to be accompanied in some animals by a dis turbance of phosphorus metabolism giving rise to lameness joint ab normalities osteoporosis and high serum phosphatase levels It has been reported also that cows under such conditions conceive with difficulty and young male bovines exhibit a complete lack of sexual interest or libido (48) The testes of these animals reveal marked damage to inter stitial cells and germinal epithelium with little or no spermatogenesis (48) Whether such effects occur in other species suffering from molyb denum toxicity is not known

2 *Other Species*

Animal species vary greatly both in their tolerance to high intakes of molybdenum and in the toxic symptoms displayed Cattle are the least tolerant, followed by sheep Horses and pigs are the most tolerant with rats rabbits and guinea pigs apparently occupying an intermediate position The minimum dietary levels of molybdenum capable of induc ing any ill effects cannot be given for any of these species on the basis of present evidence because of the number of influencing factors some known some unknown Tolerance to molybdenum is affected by the chemical form in which the molybdenum is administered (26) the copper status and copper intake of the animal the inorganic sulfate content of the diet (in sheep) the level of intake of other metals including zinc and lead (29) the intake of methionine and possibly of vitamin B_1 and the nature of the diet in other as yet unknown respects (3)

The high tolerance of horses is illustrated by their failure to show any symptoms of toxicity on teart pastures and that of pigs by the report of Davis (16) that levels of molybdenum as high as 1000 p p m were fed to these animals for a period of 90 days without any apparent ill effects The nature of the diet in respect to copper content or other constituents likely to affect molybdenum is not given but it is unlikely to have been unusually high or low Whether the great tolerance of these species is associated with a particularly low absorption of molybde num has not been determined In contrast to the results with pigs an

intake of 1000 p p m Mo, as sodium molybdate, induced gross toxic symptoms in weanling rabbits receiving a basal ration containing 2 7 p p m Mo and 16 4 p p m Cu, whereas levels of 140 p p m and 500 p p m Mo had no discernible effect (3) The effects of various levels of molybdenum on weanling rats are very variable depending upon the basal diet fed and in particular upon its copper content Thus the addition of as little as 80 p p m to a diet containing only 2 p p m of copper inhibited growth and induced a high mortality in weanling rats which could be completely overcome by raising their intake of copper with copper sulfate to the equivalent of 35 p p m (14) On the other hand 400 p p m of molybdenum as sodium molybdate produced only a slight depression of growth in weanling rats given a basal milk diet containing 6 3 p p m of copper and had no significant effect upon such animals on another type of diet containing 14 p p m of copper (29) In another experiment with young rats the addition of sodium molybdate to the diet in amounts equivalent to 5000 p p m Mo resulted in the death of all the animals within a few days, whereas intakes of 1000 p p m and 500 p p m merely inhibited growth (40) The basal diet in this case contained the remarkably high copper content of 77 p p m

On this high copper diet the addition of a further 200 p p m of copper afforded almost complete protection against the toxic effects of an intake of 400 p p m of molybdenum (40) The addition of other minerals, including iron, zinc and cobalt, was without any such effect, but dried whole liver at a level of 5% of the diet gave a very high degree of protection although its copper content was insufficient to account for its therapeutic effect The role of copper in preventing or overcoming the toxic effects of molybdenum has been established beyond doubt by many workers (3 14, 28 29 40), and recently it has been demonstrated that methionine is equally or more effective than copper or than methionine and copper together (28) (Table 18) The methionine was fed at a level of 0 6% DL methionine the molybdenum was added at a level of 0 08% (800 p p m) Mo as sodium molybdate and the copper at a level of 0 03% Cu as copper sulfate It is emphasized by these workers that the effect of the methionine resulted from an interrelationship between molybdenum and methionine and not from an over all improvement in the diet (28) The mode of action of methionine in this connection remains to be determined It may be as the authors have suggested that there is a direct complexing of the mineral by the amino acid, or it may function as a complexing agent after its conversion to homocysteine or cysteine by analogy with the effectiveness of sulfhydril compounds or

vitamin B_{12} in overcoming molybdenum toxicity in *Lactobacillus leichmannii* (151)

TABLE 18

Inhibition of Growth in Rats by Molybdenum and its Correction by Dietary Methionine and Copper (28)

Diet[a]	Mean weight change[b] (g)	Feed efficiency
1 Basal	171	2 78
2 Basal + Mo	114	3 76
3 Basal + Mo + Methionine	156	3 02
4 Basal + Mo + Cu	134	3 07
5 Basal + Mo + Methionine + Cu	162	2 90

[a] The basal diet was mineralized whole milk powder with supplements as follows Mo 0 08% fed as sodium molybdate methionine 0 6% DL methionine Cu 0 03% copper fed as copper sulfate

[b] Experimental period 6 weeks All values are mean for 6 rats (3 males and 3 females) The least difference between 2 means required for significance (t test) 1% = 24 g 5% = 17 g

Of great interest in connection with the toxic effects of molybdenum are the different symptoms displayed by different species In ruminants the outstanding feature of molybdenosis is diarrhea accompanied by loss of weight anemia and loss of coat color No sign of diarrhea has been observed in rats rabbits or guinea pigs receiving dietary intakes of this element sufficient to induce marked inhibition of growth or even death within a few days Neither has any indication of anemia been reported in rats or guinea pigs under such conditions In fact in these species retardation or failure of growth or loss of body weight accompanied by anorexia appear to be the only indications of molybdenum toxicity In young rabbits on the other hand the toxic syndrome is characterized by anorexia loss of weight alopecia dermatosis and severe anemia without diarrhea or achromotrichia In some rabbits a deformity of the front legs also develops These symptoms are indistinguishable apart from the absence of achromotrichia from those that arise in rabbits as a result of copper deficiency and the leg abnormality bears a certain resemblance to the crooked front legs produced in this species by manganese deficiency

An understanding of the underlying reasons for these species differences and also of the biochemical mechanisms involved in the profound mineral and other nutrient interrelationships that the study of

molybdenum toxicity has disclosed must await further experimentation—experimentation which includes studies of the levels of molybdenum and other elements *within the tissues* and of the activities of the enzyme systems of the tissues under a range of dietary regimes

V Molybdenum Copper Interrelations

A reciprocal antagonism between molybdenum and copper, in which the level of dietary molybdenum affects copper metabolism and the level of dietary or injected copper affects molybdenum metabolism has been demonstrated in sheep, cattle, rats, and rabbits It is likely that such an antagonism applies also in other species although this has not been investigated so far as is known The interaction between the two elements has been referred to earlier, in relation to the absorption, retention and excretion of copper and molybdenum and to the occurrence and treatment of chronic copper poisoning and molybdenosis in sheep and cattle respectively, but no over all picture of the position as at present understood has yet been presented This is attempted briefly below

The effectiveness of both oral and injected copper in controlling the pathological effects of excessive ingestion of molybdenum has been clearly established over a wide range of environmental conditions and under certain conditions not yet fully defined copper so administered exercises a limiting influence on molybdenum deposition in the liver The mechanism of this antagonistic effect is unknown and the relationship cannot yet be expressed in quantitative terms That there is a quantitative relationship can be shown by comparing the conditions under which the two diseases teart and peat scours, occur and are controlled In teart pastures high levels of molybdenum rapidly cause scouring in cattle despite a normal copper content Massive oral dosage with copper is required to counteract this amount of molybdenum In peat scours pastures a moderate excess of molybdenum causes scouring only if the pasture copper is below normal and only when the animal is depleted of copper by the low intake and by the limiting effect of molybdenum on copper storage At this intake of molybdenum control of the scouring is achieved by raising the copper content of the pastures to normal levels—levels at which severe scouring quickly develops in cattle on teart pastures These field findings have been supported by sufficient evidence from experiments with stall fed animals (15 30) to warrant the conclusion that the ratio of dietary copper and molybdenum is a factor of prime importance in determining the toxicity of any particular level of molybdenum intake It does not however imply

that other dietary factors may not exert a modifying influence on this ratio In fact this seems highly probable in view of the known fact that certain pastures with normal copper concentrations judged by standards established elsewhere induce copper deficiency symptoms such as sway back in sheep The factor or factors in these pastures responsible for this impairment of copper absorption and utilization may equally modify the effect of copper in antagonizing molybdenum

The limiting effect of molybdenum upon the retention of copper in sheep and cattle is established as unequivocally under the appropriate conditions as the opposite effect of copper on molybdenum toxicity although the mechanism of its action in this regard is just as obscure This limiting effect is apparently an exceedingly complex process in which dietary factors other than copper known and unknown are powerfully involved The demonstration that molybdenum influences copper metabolism in this way only in the presence of adequate intakes of inorganic sulfate (19 20) was a big step forward and there is no doubt that many of the apparently conflicting results reported can be explained on the basis of different levels of inorganic sulfate in the diets employed It is however not the whole story The nature of the effect of molybdenum on copper metabolism is also influenced greatly by the level of molybdenum intake and by the copper status of the animal In the presence of adequate dietary sulfate molybdenum can both deplete the copper concentration of the tissues and blood in sheep and maintain or increase these concentrations depending upon the intake Moreover it can precipitate severe symptoms of copper deficiency while maintaining the concentrations of copper in the blood and tissues at normal or near normal levels Thus Marston (34) reported the results of two experiments in which high doses of molybdenum (50 and 100 mg Mo per day as molybdate) were administered to sheep Where the sheep were grazed on pastures normal in copper content the molybdenum treatment resulted in decreased copper concentrations in the liver Where the sheep were grazed on the copper deficient pastures of the calcareous littoral of South Australia the effects of the high doses of molybdenum were strikingly different By comparison with controls receiving no added molybdenum there was a significant *decrease* in the rate of depletion of copper from the liver the maintenance of significantly *higher* concentrations of copper in the blood and the precipitation of the symptoms of copper deficiency *"sooner and in most flagrant form"*

The value of these important observations has been greatly increased by the studies of Dick (18) with pen fed sheep given copper sufficient

diets to which varying amounts of inorganic sulfate and molybdenum were added These showed very convincingly that the influence of molybdenum on blood and liver copper concentrations depends upon the magnitude of *both* molybdenum and sulfate intakes At high levels of molybdenum (60–90 mg Mo per day) the concentration of copper in the blood is greatly increased and liver copper values do not fall so long as the diet contains sufficient inorganic sulfate The amount by which the level of copper in the blood rises was found to be dependent upon the molybdenum intake within the range 15–90 mg/day when the sulfate intake is constant and upon the sulfate intake when the molybdenum level is constant within the same range It was found further that the sheep receiving the high molybdenum high sulfate diets quickly developed typical copper deficiency lesions in their wool despite the high blood and tissue copper concentrations

It has been postulated that the coexistence of symptoms of copper deficiency with normal concentrations of copper in the blood and tissues i e the physiological copper deficiency which occurs at high intakes of molybdenum and sulfate results either from the molybdenum fixing the copper in a form which is not available for physiological functions or from the molybdenum antagonizing the copper containing enzymes (18, 34) It can be postulated further that the rise in blood copper concentrations which occurs under these conditions results from the mobilization of copper reserves in an attempt to overcome the "physiological copper deficiency just described The fact that liver copper values do not fall when sheep are subjected to these high molybdenum, high inorganic sulfate conditions but do fall at lower molybdenum intakes, suggests that the normal mechanisms of copper excretion as well as utilization are interfered with at the higher levels of molybdenum but not at the lower (18) The validity of these hypotheses can only be determined and the elucidation of the whole problem of molybdenum copper interrelationships achieved by a great deal more experimentation which includes a range of closely controlled copper molybdenum and inorganic sulfate intakes by the animal and very complete and detailed studies of the distribution of copper and molybdenum in its tissues, fluids and excretions at each of these intakes

VI Molybdenum and Xanthine Oxidase

The identification in 1953 of xanthine oxidase as a molybdoflavoprotein (42 44) implies that molybdenum is an essential element in the diet of animals at least to the extent that xanthine oxidase is concerned with essential metabolic processes Ten years earlier Teresi

Elvehjem and Hart (46), prompted by previous findings that this element is widely distributed in plant and animal tissues and is required by various forms of plant life for normal growth and development attempted to determine whether it is an essential nutrient for animals They fed groups of weanling rats for a period of six weeks on a diet of goat s milk mineralized with iron copper and manganese with and without added molybdenum Comparable growth was shown by litter mates on this diet whether the molybdenum was added or not As the rats consumed some 40 ml of milk daily containing 0 5 μg Mo it was concluded that if the young rat requires molybdenum for growth its requirement must be less than 0 5 μg /day This represents approximately 0 01 p p m of the dry diet No tissue xanthine oxidase assays were of course made in this study

About this time it was shown that riboflavin (5) and protein (35) deficiencies decreased the concentration of liver xanthine oxidase in the rat. Subsequent investigations by Westerfeld and Richert (50) established that xanthine oxidase was present in small amounts in the lung spleen kidney and skin and in large amounts in the liver and small intestine that liver and intestinal xanthine oxidase was markedly depleted by a low protein purified diet that *the amount remaining was adequate to maintain a normal excretion of uric acid and allantoin* and that the levels achieved with a purified diet containing adequate protein and riboflavin were only 75% or so of the values obtained with diets containing natural foodstuffs such as liver or milk It was postulated that these foodstuffs contained some factor designated the xanthine oxidase factor or XOF responsible for this effect (51) and that this factor was probably related to the unidentified component of the prosthetic group of xanthine and aldehyde oxidases (6) Two groups of workers then tackled the problem of the isolation and identification of the xanthine oxidase factor by a series of fractionation procedures applied to liver residues (42) and soy flour (44) It was shown in each case that the active component in these materials responsible for maintaining the xanthine oxidase content of the tissues was molybdenum The response of intestinal xanthine oxidase in the rat to increasing amounts of molybdenum in the purified diets used is shown in Fig 14

This finding was immediately followed by the demonstration that molybdenum is a nondialyzable component of xanthine oxidase itself (31 39) and that this enzyme contains two molecules of flavin adenine dinucleotide and one atom of molybdenum per molecule and has a molecular weight of about 230 000 (31) Subsequently it was shown that

xanthine oxidase contains iron as well as molybdenum, that iron flavin and molybdenum occur in a ratio of 8 2 1 and that the minimum molecular weight of the enzyme is approximately 320 000 (43)

It should be emphasized that in none of the experiments so far reported has the depletion of liver or intestinal xanthine oxidase been accompanied by any detectable disability in the experimental rats nor has the addition of molybdenum to the xanthine oxidase depleting diets

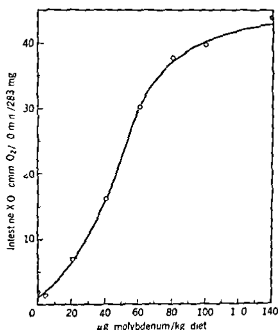


FIG 14 The response of intestinal xanthine oxidase in the rat to increasing amounts of molybdenum in the diet (Westerfeld and Richert 53)

resulted in any improvement in their growth or metabolism Until such effects are produced molybdenum cannot be said to have been unequivocally established as an essential dietary item in this or any other species It is entirely possible for instance that uric acid can be formed by a mechanism which does not involve xanthine oxidase On the other hand it may be that the amount of xanthine oxidase in the rat far exceeds the amount required to fulfil its normal function and that significant functional defects will arise when the amounts of the enzyme are still further reduced by the development of still more molybdenum deficient diets De Renzo and co workers (42) found that diets containing 0 02 p p m molybdenum supplying 0 2–0 3 µg Mo per rat per day were sufficient to produce a "saturation level of xanthine oxidase This implies a *maximum* daily requirement of molybdenum of this order or about one half of that shown earlier to be sufficient for the growth of young rats (46) The basal diets of Westerfeld and Richert also contained

about 20 μg Mo per kg or 0.02 p p m It seems that diets appreciably lower than this in molybdenum are necessary and that they must be fed for sufficiently long periods to deplete the animal's molybdenum stores, particularly those in the bones, before a clear claim can be made that this element is a dietary essential

These findings with rats raise a number of interesting questions concerning the effects of very low molybdenum diets on grazing sheep and cattle and as to whether some pastures may be so low in molybdenum that they impose some physiological disability on the grazing animal. The significance of pastures of low molybdenum content in favoring the accumulation of copper in the liver and other tissues of sheep and therefore in the occurrence of chronic copper poisoning in these animals was discussed in the section on copper toxicity in Chapter 3. The only other condition which, so far as is known, can even remotely be related to molybdenum insufficiency is that of the occurrence of a high incidence of xanthine renal calculi in sheep grazing on the Moutere Hills pastures in New Zealand (25). The soils of these hills have subsequently been shown to be very low in molybdenum compared with neighboring soils where a similar high incidence of xanthine calculi had not been observed (32) but pasture analyses for molybdenum have not been obtained which would enable molybdenum deficiency to be implicated as a causal factor in this condition. Nor have the tissues of affected animals yet been examined for molybdenum. Moreover, there is evidence that the incidence of such calculi in sheep grazing these pastures decreased well before molybdenum treatment of soils and pastures was applied to improve plant productivity (4). It is obvious that a great deal more study will be necessary before this condition in sheep can be considered a manifestation of molybdenum deficiency.

VII Distribution of Molybdenum in Foods

No comprehensive investigations of the concentrations of molybdenum in human foods or estimates of molybdenum intakes from average human dietaries appear to have been carried out. Ter Meulen (47) reported beans and peas to be relatively rich in molybdenum (3–9 p p m) with whole cereal grains next in order as a group containing 0.2–0.6 p p m and various fruits and vegetables containing "only minute traces". Subsequent investigation has confirmed the relative richness of legumes and of cereal grains and has shown that the molybdenum in the latter is concentrated to some extent in the germ and possibly the bran (9). In a more extensive recent study Westerfeld and

Richert (52) found legumes, cereal grains, and some green leafy vegetables to be good sources of molybdenum and fruits berries and most root or stem vegetables to be relatively poor sources Liver, kidney and spleen were the only animal tissues examined which were considered "good" sources of molybdenum The variation among different samples of the same classes of foods was so great however, that classification in this way is of doubtful value Thus the range of values reported for the total molybdenum concentrations found by these workers was 0 25–4 69 p p m for the leguminous seeds 0 12–1 14 p p m for the cereal grains, and 0 14 and 0 54 p p m for two samples of onions Westerfeld and Richert also assayed these and various other foods for available molybdenum by determining the intestinal xanthine oxidase response to their incorporation into an otherwise purified molybdenum deficient diet. Comparison of these assay results with the total molybdenum determined chemically showed that 50–100% of the dietary molybdenum was "available" for this biological response

The molybdenum concentration in plants varies with the species and with the soil type Crucifers are relatively rich in this element and are subject to a molybdenum deficiency disease on certain soils (e g "whiptail in cauliflowers) in which the leaves may contain markedly subnormal concentrations of molybdenum (41) Significant differences among pasture species in molybdenum content have been demonstrated even when these are grown on the same soil type (23) but the molybdenum status of the soil and its pH are the main determinants of the concentration of this element in plants Molybdenum is not readily absorbed by plants from acid soils, and liming of such soils or the addition of small quantities of molybdate or both, are very effective means of raising the molybdenum content of pasture plants The importance of soil conditions is evidenced by the fact that molybdenum contents of pasture species have been reported varying from less than 0 1 p p m in some areas to over 25 p p m on the alkaline teart soils which occur in restricted areas in England and elsewhere

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The first serious scientific work on any of these wasting diseases began in New Zealand at the end of the 19th century It was soon established that bush sickness was not due to either an infectious or a toxic agent and a mineral deficiency or excess was postulated to explain the condition A long series of investigations by Aston and co workers then led to the claim that bush sickness was due to a deficiency of iron (15) This claim was based upon the occurrence of anemia in affected animals the low iron content of "bush sick" soils and pastures compared with those of healthy areas and, most compelling of all the effectiveness in the cure or prevention of the disease, of dosing stock with crude iron salts or ores

Within a few years the iron deficiency theory of Aston had become generally accepted and appeared to be supported by the finding that similar conditions in sheep and cattle, in several different parts of the world, responded similarly to treatment with crude iron compounds (45) In fact long after this theory had been disproved occasional reports appeared suggesting that certain debilitating maladies of grazing sheep and cattle were manifestations of a deficiency of iron in the soils and pastures of the affected areas The virtual impossibility of iron deficiency ever arising in grazing stock has been discussed previously (Chapter 2) and need not be repeated here

Subsequent work in New Zealand demonstrating insignificant differences in iron content between certain bush sick and healthy pastures (127) and little correlation between the curative effect of various iron compounds and their iron content (67), threw some doubt on the iron deficiency theory but indisputable evidence against it was provided by Filmer and Underwood (51, 54 148) in their investigations of a wasting disease of sheep and cattle (enzootic marasmus) in certain localized areas in Western Australia These workers became suspicious of the extremely high doses of iron compounds required to cure this condition and like the New Zealand workers just mentioned observed little correlation between the size of an effective dose and the amount of iron supplied They found further that the livers and spleens of affected animals contained excessive stores of iron and that whole liver was curative in doses which supplied insignificant amounts of iron An iron *free* extract of one of the curative compounds (limonite $Fe_2O_3H_2O$) was then prepared which was just as potent as whole limonite It was clear that iron deficiency was no longer tenable as the cause of enzootic marasmus and that this disease was probably due to a deficiency in the herbage of some trace element which occurred as a contaminant of the iron compounds used In search of this element Underwood and

Filmer (151) divided an iron free extract of limonite into chemical fractions and found the potency to reside in the zinc group of elements which included zinc manganese nickel and cobalt Following some misleading tests with nickel suggested by the relatively large amount of this element in this fraction various combinations of the four elements of the zinc group were tested on affected animals which showed clearly that the potency of the iron compound lay in the cobalt which it contained Normal growth and health of sheep and cattle on the affected pastures were sustained by the administration of minute oral supplements of cobalt chloride alone Subsequently it was shown that the soils and pastures of the affected areas contained much lower concentrations of cobalt than those of healthy areas and that the livers of affected animals contained subnormal levels of this element (152)

While the New Zealand and Western Australian investigations were proceeding studies of coast disease of sheep occurring on the calcareous sandy dunes of South Australia were being carried out The possibility that this disease was due to an absolute deficiency of a mineral element or to a deficiency induced by the high consumption of calcium carbonate from the environment was early recognized Supplements of phosphorus and of copper were completely ineffective and the particular iron compounds in the inadequate doses used produced only a transitory improvement in the condition of "coasty" sheep (109) A mineral mixture supplying small amounts of iron copper boron manganese cobalt nickel zinc arsenic bromine fluorine and aluminum on the other hand induced growth and reproduction in ewes on coasty country comparable with that of ewes maintained on sound pastures (109) The fact that coast disease is accompanied by a progressive anemia and the earlier finding of Waltner and Waltner (156) that cobalt stimulates hematopoiesis in rats led to the suggestion that cobalt might be the particular element responsible for the beneficial effects of the mineral mixture Preliminary experiments by Lines (97) and Marston (100) showed that the administration of 1 mg of cobalt per day by mouth resulted in a dramatic improvement in the appetite body growth and hemoglobin levels of coasty sheep This important finding was made several months prior to the similar finding of Underwood and Filmer (151) Subsequent experiments by Marston and co workers (109) showed that treatment of coast disease with cobalt alone gave highly variable results and that the progressive development of anemia could not be prevented by these means Further supplementation with copper was found to be completely successful and it was concluded that this disease is the result of a dual deficiency of cobalt and copper (109)

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Immediately following the publication of the Australian results with cobalt cobalt supplements were found to be equally effective in the cure and prevention of bush sickness in New Zealand (12), salt sick in Florida (117), pining in various parts of England and Scotland (40) and nakuruitis in Kenya (7) all of which had been earlier shown to respond to massive doses of iron compounds Within a few years a number of rather similar conditions in sheep and cattle grazing certain very localized areas in many parts of the world, including Canada the United States Ireland Norway Sweden, Denmark and Estonia had also been shown to be due to a deficiency of cobalt in the soils and herbage and to respond to cobalt therapy References to these are given in reviews by Owen (121) and Marston (102) It seems highly likely that there are other areas in the world where cobalt deficiency in grazing ruminants exists although this may not necessarily be in the acute form described below

2 *Manifestations of Cobalt Deficiency*

A striking feature of cobalt deficiency in both sheep and cattle is the absence of any symptoms which can be regarded as specific for this element The appearance of a severely cobalt deficient animal is one of extreme emaciation and listlessness indistinguishable from that of a starved animal (Fig 15), except that the visible mucous membranes are blanched and the skin is usually pale and fragile The emaciation or wasting of the musculature (marasmus) results from the failure of appetite which is an early and conspicuous symptom of the condition and the paleness of the mucous membranes and skin from the profound anemia which usually develops progressively with the severity of the cobalt deficiency

The intensity of the symptoms shown, and the rate at which they develop, depend primarily upon the concentration of cobalt in the herbage consumed and upon the age and previous history of the animal Young growing lambs and calves exhibit the deficiency syndrome more quickly and severely than adults under the same conditions and in some areas and some seasons only young stock are affected The seasonal (climatic) conditions and botanical nature of the pastures may also affect the incidence and severity of cobalt deficiency, especially in marginal or incipiently deficient areas (90) The nature of the seasonal factors which affect the cobalt status of pastures presumably through a variable uptake of cobalt by plants from the soil is little understood except through their influence on botanical composition Grasses usually

carry lower concentrations of cobalt than legumes or "herbs" under the same conditions (90 143)

It is not surprising in view of the factors influencing cobalt def just outlined that the syndrome varies from the acute and fat dition described earlier through a series of less acute stages to



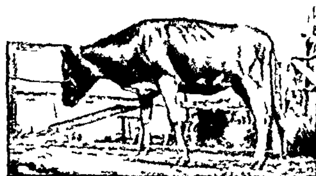


Fig 15 Above a heifer showing severe cobalt deficiency Below the animal several weeks after treatment with cobalt (Photo by Dr J F Fil

ill defined and often transient state of unthriftiness which ca be diagnosed with certainty as cobalt deficiency by the rapid re which follows dosing of the animals with cobalt When sheep o are turned on to typical cobalt deficient terrain or are stall cobalt deficient herbage there is however a characteristic re At first they grow or thrive normally This period may last for months depending upon their age and previous history There

followed by extreme inappetence, weakness, rapid wasting or loss of condition accompanied by a progressively severe anemia culminating in death (54, 109) It should be pointed out that these are extreme consequences resulting from acute deficiency and that milder forms especially in adult stock characterized by unthriftiness suppression or diminution of lactation and the birth of weak lambs or calves which rarely survive more than a few weeks, are common in many affected areas (51, 109)

The body of severely affected animals presents on autopsy a picture of extreme emaciation with often a total absence of body fat The liver is fatty, the spleen hemosiderized, and there is a hypoplasia or aplasia of erythrogenic tissue in the bone marrow The red cell count and the hemoglobin concentrations are always low in affected animals in the field The levels may be only 30% of normal, or even lower, according to the degree of deficiency There is some disagreement on the nature of this anemia Filmer (51) found it to be normocytic and hypochromic in lambs and microcytic and hypochromic in calves, with anisocytosis and marked poikilocytosis but without either polychromasia or punctate basophilia Smith *et al* (138) report a normocytic and normochromic anemia in lambs whereas Marston (102) reports the condition in sheep as a profound macrocytic anemia with marked poikilocytosis and polychromasia' Detailed studies of the morphological characteristics of the blood in cobalt deficiency do not appear to have been undertaken elsewhere There is general agreement however that it is not the anemia which is responsible for the general symptoms of cobalt deficiency Sheep fed cobalt deficient rations in pens occasionally die of inanition before becoming severely anemic Inappetence and marasmus invariably precede any considerable degree of anemia and the first discernible response to cobalt feeding is an almost immediate and rapid improvement in appetite and body weight followed by a delayed although often equally dramatic improvement in the blood picture Some workers have actually reported a small temporary fall in hemoglobin and in red cell numbers coincidental with a dramatic improvement in appetite and appearance immediately following cobalt treatment (16, 51)

3 *Cobalt Requirements*

The quantity of cobalt necessary to fulfil the nutritional requirements of ruminants is extremely small—certainly less than one tenth of their requirement for copper and apparently very similar to their requirement for iodine Normal pastures and fodders vary greatly in cobalt content

but mostly lie within the range 0.1–0.3 p.p.m. on the dry basis. Deficient pastures generally contain only 0.03–0.05 p.p.m. but values as low as 0.01 p.p.m. have been recorded. From extensive studies of New Zealand pastures (10, 112) it appears that a cobalt concentration of 0.07 p.p.m. in the dry matter can be considered as the minimum compatible with satisfactory growth and health of sheep and cattle. The position is very similar in Western Australian pastures according to Underwood and Harvey (152). The somewhat higher minimum level of 0.1 p.p.m. is suggested by Stewart (140) on the basis of Scottish experience.

Sheep grazing deficient pastures containing 0.02–0.04 p.p.m. of cobalt were quite early shown to respond to daily treatment with cobalt salts supplying as little as 0.05 mg. Co daily but optimum growth and health was only obtained with a daily supplement of 0.1 mg. (55). A more precise assessment of the minimum requirements of sheep has since been made by Marston and Lee (102). These workers found that a supplement of 0.05 mg. cobalt daily, administered by mouth thrice weekly, was just sufficient to provide the requirements for a period of three years of sheep grazing herbage containing 0.02–0.03 p.p.m. of cobalt. This indicates that a total ingestion of 0.07–0.08 mg. Co per day will completely fulfill the requirements of the sheep and that a diet containing about 0.08 p.p.m. in the dry matter will therefore suffice for this species. It will be noted that this figure lies between the minima set by the New Zealand and Scottish workers. The minimum cobalt requirement of bovines is not so precisely known but field evidence indicates that this must be very similar, in terms of cobalt concentration in the fodder, to that of sheep. Daily supplements of 0.3–1.0 mg. cobalt have been found adequate for the maintenance of normal growth and health of cattle grazing typical cobalt deficient pastures (55).

4. *Prevention and Treatment of Cobalt Deficiency*

Cobalt deficiency in ruminants can be prevented or overcome either by direct administration of cobalt to the animal, by injections of vitamin B_{12}, or in most areas by treatment of the soils or pastures with cobalt-containing fertilizers. Treatment with vitamin B_{12}, to be discussed later, seems hardly likely to become of great practical significance in the control of cobalt deficiency for reasons of cost and inconvenience. The provision of adequate supplies of cobalt either directly or through the pastures so that the animals can produce their own requirements of vitamin B_{12} is therefore the most economic and widely practised form of control.

a. *Direct Administration of Cobalt*. Early in the investigations of cobalt deficiency it was found that to be fully effective animals must

be dosed frequently with cobalt salts For the best results dosing at not more than weekly intervals is necessary Correspondingly larger doses given at fortnightly intervals are helpful but do not prevent the onset of deficiency symptoms (102) Drenching at monthly intervals with much larger doses of cobalt than the monthly equivalent of the daily dose will prevent obvious signs of deficiency in sheep on typical cobalt deficient pastures but it will not produce optimum results (52) Lee (91) states that cobalt deficiency is completely controlled in sheep by drenching once each week with 7 mg cobalt whereas the official New Zealand recommendation is that cobalt doses for deficient sheep should contain the equivalent of 2 mg cobalt and be given twice each week

This necessity for frequent and regular dosing with cobalt contrasts greatly with the position with other trace elements such as iron and copper These elements are readily stored in the tissues especially in the liver, during periods of abundant intake and these stores are normally available to the body to help tide over periods of inadequate intake The situation is quite different with cobalt The animal body has a very limited capacity to store this element, which is quickly and almost completely eliminated from the body Moreover, tissue cobalt does not readily pass to the alimentary tract where its action is exerted Frequent dosing by mouth is therefore essential to maintain the required concentrations of cobalt within the tract particularly in the rumen itself

To be completely effective cobalt must not only be administered frequently but it must also be given by mouth Injection into the blood stream is useless (Fig 16) even though injections have been shown to increase, and if repeated, maintain, the concentration of cobalt in the liver and blood ten fold (21 104 125 126a) The ineffectiveness of injected cobalt is due to the fact that it does not reach the site of action i e the rumen or the rumen and the reticulum and the omasum Some improvement in the condition of deficient animals has been observed where very large amounts are injected (84 126a) but this can possibly be explained by small amounts entering the rumen in the saliva or through the rumen wall It has been shown that injected radioactive cobalt does not appear in the rumen reticulum or omasum except when very large amounts are injected and even then only in extremely small quantities (38) Direct placement of cobalt salts into the abomasum or the duodenum by means of abomasal and duodenal fistulas is not as effective as cobalt given by mouth but is certainly of some benefit (125) In this case definite evidence was obtained that cobalt administered in these ways reaches the rumen The concentrations of cobalt in the rumen liquor of sheep receiving cobalt directly into the abomasum or duodenum

were three or more times greater than in the rumen of lambs receiving no cobalt (125). With the abomasal placements this can possibly be explained on the basis of oral diffusion but with the duodenal placements the more likely possibilities are those mentioned in relation to large intravenous injections, namely, entrance via the saliva or through the rumen wall.

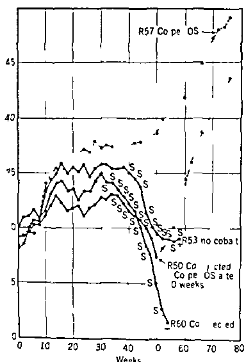


FIG 16. Typical responses of ewes on cobalt deficient pastures. R53 untreated. R57 1 mg Co daily *per os*. R60 1 mg Co daily injected. R50 1 mg Co daily injected plus 1 mg Co daily *per os* after 50 weeks when the deficiency symptoms were in a terminal stage. S denotes recognizable deficiency symptoms. (Marston and Lee 104)

b *Treatment of Pastures.* The necessity for frequent drenching of individual animals with cobalt salts led to the investigation of other less tedious and more economical means of control. The provision of cobalt containing salt licks or supplements has some advantages and is practised in some areas but it is not always easy to ensure a regular and adequate supply of cobalt to all animals by this method. The most economic and most widely practised means of ensuring continuous and adequate supplies of cobalt in many deficient areas is through treatment or "topdressing" of the pastures with fertilizers with which a proportion of cobalt salts or ores has been mixed ("cobaltized" fertilizers). In this way the concentration of cobalt in the pastures can be raised to normal levels for extended periods without affecting the growth of the plants. As little as 4–5 oz of cobalt sulfate per acre applied annually or biennially on many deficient pastures (11, 129) and a single topdressing with 20

oz of cobalt sulfate, has maintained pasture levels of cobalt and the health of grazing stock at a normal level for a period of 6 years in some parts of New Zealand and for 3 years in others (4) Aerial topdressing with solutions of cobalt sulfate at this level has proved fully effective in controlling cobalt deficiency on rugged hill grazings not accessible to farm machinery (4) The effectiveness of these treatments depends primarily upon the soil types but also upon the species of plants which vary in their capacity to absorb cobalt from the soil On the calcareous sand dunes of South Australia treatment of the soil or herbage with cobalt is much less effective owing to the relatively low uptake of the added cobalt from soils of this type

c *Treatment with Liver Liver Extracts and Vitamin B_{12}* Very early in the experimental study of cobalt deficiency the effect of treatment of affected animals with liver was tried Both fresh and dried liver, in the large doses of 200 g and 40 g /day respectively were found to be highly curative when fed to lambs or calves (51) although it was later shown that these doses supplied insufficient cobalt to account for their beneficial effects (55) Liver ash in equivalent amounts was found to be ineffective This finding which was subsequently confirmed in New Zealand (53) led Filmer and Underwood (55) to suggest prophetically that

the potency of liver may be due to the presence of a stored factor and that cobalt may function through the production of this factor within the body "

These results with liver could not be repeated by Marston and Lee (105) with sheep on cobalt deficient pastures and Becker and Smith (19) were also unsuccessful with oral doses of liver preparations administered to cobalt deficient lambs in pens Whether this disagreement can be resolved in terms of the amounts or composition of the liver fed is not known, but evidence that antipernicious anemia extracts of liver are curative of cobalt deficiency when *injected* was obtained by these latter workers (19) They separated a potent liver extract into 24 fractions by the countercurrent distribution technique and found a high correlation between the vitamin B_{12} activity of these fractions and the appetite, weight and hemoglobin responses in cobalt deficient lambs (139) This led to a re evaluation of the curative effects of pure vitamin B_{12} which earlier studies had shown to be ineffective in the doses then used (21 104) When Smith *et al* (139) injected doses of 150 μg or more of vitamin B_{12} twice weekly into affected lambs completely favorable responses were obtained This important finding as will be shown was subsequently confirmed and extended in several laboratories (2 6 105)

The recognition in 1948 that vitamin B_{12} is a cobalt containing complex (137a) stimulated immediate interest in a possible relationship between this compound and cobalt deficiency in ruminants It had already been shown that relatively large amounts of vitamin B_{12} are normally present in the rumen contents (61 68 73a) and the rate of its synthesis there and in the lower levels of the gastrointestinal tract was found to be greatly reduced in the cobalt deficient animal (1 61 68 73a) However, the first attempts to overcome cobalt deficiency in sheep with vitamin B_{12} administered either parenterally or *per os* were unsuccessful as was indicated previously The doses used in these preliminary investigations (1 μg of vitamin B_{12} or its equivalent per day to lambs (21) and 15 μg per week to sheep (104) were based upon the amounts used successfully in the treatment of human pernicious anemia patients It is now known that the metabolic demands of the ruminant for this vitamin are very much greater than those of man and probably of other nonruminant species

The minimum requirement of injected vitamin B_{12} to prevent all signs of deficiency and promote full growth and health in ruminants consuming severely cobalt deficient fodder has not yet been determined precisely but in lambs it appears to be very close to 100 μg/week (6 89) Marston and co workers (105 108) used arbitrary doses of either 300 μg/week or 50 μg day which is practically identical with that used originally by Smith Koch and Turk (139) At this level of injection immediate and rapid remission of the symptoms of cobalt deficiency is obtained in sheep (Fig 17) In fact both the blood dyscrasia and the general condition and appetite responded slightly more rapidly to treatment with this quantity of vitamin B_{12} than to dosing with 1 mg Co per day Anderson and Andrews (2) found that unthrifty lambs grazing a cobalt deficient pasture responded at least equally well to vitamin B_{12} injected at the much smaller dose of 100 μg/week as they did to cobalt dosed orally at the rate of 7 mg a week (Fig 18) A single massive injection of 1000 μg vitamin B_{12} produced only a brief response in such lambs in contrast to the position in humans where single injections of this order secure remission of pernicious anemia for periods ranging from 4 to 12 months (155) Some response to oral vitamin B_{12} at the very high dose levels of 1000 μg week was obtained by Andrews and Anderson (6) but this response was appreciably less than the responses to injected vitamin B_{12} or oral cobalt (Fig 18) Vitamin B_{12b} (hydroxocobalamin) is as effective as vitamin B_{12} itself in curing cobalt deficiency in lambs when injected at the rate of 100–125 μg/week (89)

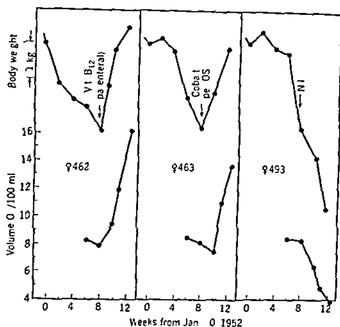


FIG 17 Typical body weight and hemoglobin responses of ewes to injected vitamin B_{12} and oral cobalt (Marston and Lee 105)

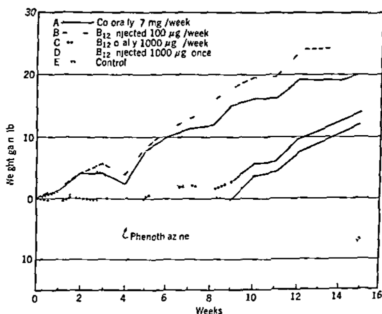


FIG 18 Effects of various treatments with oral and injected vitamin B_{12} and oral cobalt on lambs grazing cobalt deficient pasture (Andrews and Anderson 6)

5 *Mode of Action of Cobalt*

Abundant evidence is now available from which it can be concluded that ruminants have only an indirect need for cobalt and that this element functions primarily if not wholly through its action in promoting the growth of those microorganisms in the rumen whose function or one of whose functions is to synthesize the considerable needs of the host animal for vitamin B_{12} Only when their nutrient medium the rumen liquor contains a sufficient concentration of cobalt can these organisms proliferate in sufficient numbers to provide the body needs for this vitamin and prevent the development of the characteristic syndrome of cobalt deficiency Cobalt deficiency in ruminants is therefore in effect a vitamin B_{12} deficiency

The evidence in support of this conclusion some of which has already been presented can be summarized as follows (a) Ruminants are the only species in which cobalt deficiency has been demonstrated (b) Cobalt must be ingested if it is to be fully effective (Fig 16) or it must be placed further along the alimentary tract in sufficient quantities and in such a position that it can pass to the rumen reticulum and omasum in appreciable amounts (125) (c) High concentrations of cobalt *per se* can exist in the blood and tissues following injection without preventing symptoms of cobalt deficiency (d) The total amounts and concentrations of cobalt in the rumen contents and in the microbial fraction of the rumen contents are very much lower in the cobalt deficient than in the normal animal (125 146) (e) There is a marked diminution in the numbers of bacteria in the rumen and significant changes in the types of organism present in the cobalt deficient animal (61) These bacterial changes are not the result of lowered feed intake as such and can be restored to normal by cobalt feeding but not by *cobalt injection* (f) *The rumen contents of normal animals* are a rich source of vitamin B_{12} but the concentration of this vitamin is very greatly reduced in cobalt deficiency (61 68 73a 126) (g) The blood and the liver of cobalt deficient sheep contain very much lower levels of vitamin B_{12} than the blood and liver of normal sheep or of sheep given adequate cobalt but whose feed intake is limited to that of cobalt deficient animals (74) (Table 19) The urinary and fecal excretion of vitamin B_{12} is also very much lower in cobalt deficient than in cobalt fed sheep (44) (h) Cobalt deficient lambs and sheep respond dramatically and completely to injection of vitamin B_{12} or vitamin B_{12b} in doses (100–300 μg/week) which are very large in comparison with those that are effective in human pernicious anemia At these dose levels there are

TABLE 19

THE VITAMIN B_1 ACTIVITY OF THE BLOOD LIVER AND RUMEN INGESTA OF COBALT DEFICIENT AND COBALT FED SHEEP (74)

	Cobalt deficient		Cobalt sufficient (full fed)		Cobalt-sufficient (limited fed)	
	Number of sheep	Mean ± S D	Number of sheep	Mean ± S D	Number of sheep	Mean ± S D
Whole blood						
mg /ml	16	0.47 ± 0.11[a]	6	2.3 ± 0.6	3	4.3 ± 1.5
Liver						
μg /g moist weight	9	0.055 ± 0.015[c]	6	0.93 ± 0.26	3	1.24 ± 0.20
Rumen ingesta[b]						
μg /g dry weight	4	0.09 ± 0.06[c]	5	1.3 ± 0.4	3	1.3 ± 0.9

[a] Taken early in cobalt deficiency

[b] It should be noted that the values for rumen ingesta are not directly comparable with those for liver and blood since the latter owing to the method of assay include a high proportion of vitamin B_1 like compounds not present within the tissues (see text)

[c] Taken from severely cobalt deficient animals

indications that the responses in appetite, weight gains and hemoglobin are slightly more rapid than with oral cobalt

These findings leave no doubt that the symptoms of cobalt deficiency in ruminants arise as a result of a shortage of vitamin B_1 but they do not prove that vitamin B_1 itself is the only cobalt containing compound of importance to the ruminant They also leave many questions still to be answered such as the relation of vitamin B_1 to appetite and the reason for the relatively high requirements of the ruminant for this vitamin Furthermore although the conclusion that cobalt functions merely to stimulate the proliferation of those microorganisms within the rumen that produce vitamin B_1 is a logical deduction from existing facts it has not yet been supported by any direct microbiological evidence in which particular types or species of organisms have been implicated in vitamin B_1 synthesis and shown to be reduced in number in the cobalt deficient animal or to increase with cobalt ingestion However the cobalt deficiency state is not accompanied by any over all impairment of digestibility (19) which suggests that those organisms in the rumen responsible for carbohydrate breakdown are not reduced in number or effectiveness Obviously a more thorough investigation of the microbiological aspects of the whole problem of cobalt deficiency is badly needed

The possibility that cobalt is concerned in the ruminant with the formation of cobalt containing compounds other than vitamin B_1 is suggested by some recent findings whose significance is difficult to evaluate at present It has been established that only a small proportion of the vitamin B_1 activity (by *B coli* and *L leichmannii* assay) of the rumen contents of calves and sheep is due to vitamin B_1 itself (cyanocobalamin) As much as 90% of this activity may be due to other compounds whose exact chemical nature remains to be determined but which are inactive in the animal whereas the vitamin B_1 activity of the *livers* of cattle and sheep is due almost entirely to vitamin B_1 itself (58 126) The nature and potency of these compounds in relation to vitamin B_1 are discussed in a later section For the present it can be stated that true vitamin B_1 can be assayed by means of the protozoon *Ochromonas malhamensis* These findings led Ford Kon and Porter 58 126) to put forward the hypothesis for which there is yet no clear proof that cobalt has a double role in the ruminant This is for the production of vitamin B_1 which is clearly essential for the normal metabolism of the animal and for the formation of the other vitamin B_1 like compounds which have no B_1 activity within the tissues of the

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THE VITAMIN $B_{1_}$ ACTIVITY OF THE BLOOD LIVER AND RUMEN INGESTA OF COBALT DEFICIENT AND COBALT FED SHEEP (71)

	Cobalt deficient		Cobalt sufficient (full fed)		Cobalt sufficient (limited fed)	
	Number of sheep	Mean ± S D	Number of sheep	Mean ± S D	Nt mber of sheep	Mean ± S D
Whole blood mg /ml	16	0 47 ± 0 11[a]	6	2 3 ± 0 6	3	4 3 ± 1 5
Liver µg /g moist weight	9	0 055 ± 0 015[c]	6	0 93 ± 0 26	3	1 24 ± 0 20
Rumen ingesta[b] µg /g dry weight	4	0 09 ± 0 06[c]	5	1 3 ± 0 4	3	1 3 ± 0 9

[a] Taken early in cobalt deficiency

[b] It should be noted that the values for rumen ingesta are not directly comparable with those for liver and blood since the latter owing to the method of assay include a high proportion of vitamin B_{12} like compounds not present within the tissues (see text)

[c] Taken from severely cobalt deficient animals

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Rumen ingesta[b] μg /g dry weight	4	0 09 ± 0 06[c]	5	1 3 ± 0 4	3	1 3 ± 0 9

[a] Taken early in cobalt deficiency

[b] It should be noted that the values for rumen ingesta are not directly comparable with those for liver and blood since the latter owing to the method of assay include a high proportion of vitamin B_{1-} like compounds not present within the tissues (see text)

[c] Taken from severely cobalt deficient animals

of vitamin B_1 although the precise mechanism involved is equally obscure but the relation of vitamin B_1 to the inappetence which is such a dominant feature of cobalt deficiency in these species and which responds so dramatically to either oral cobalt or parenteral vitamin B_{12} is not understood at all The most likely explanation would seem to lie in some derangement of the fermentation process consequent upon the depression in numbers and changes in types of microorganisms present in the cobalt deficient rumen This could lead to the formation or to the accumulation of abnormal breakdown products due to a reduced rate of passage of such products along the alimentary tract (141)

6 *Cobalt and Phalaris Staggers*

"Phalaris staggers is the popular name given to a disease of sheep and cattle which occurs when these animals are restricted to certain grazings consisting predominantly of the perennial grass *Phalaris tuberosa* In most areas where this grass is prevalent the malady is unknown The disease affects animals of any age and is characterized clinically by marked incoordination of gait (staggers) generalized muscular tremors and rapid breathing and pounding of the heart which are particularly apparent when the animals are driven or disturbed Chronically affected animals repeatedly fall and experience great difficulty in rising Frequently the fetlocks remain knuckled over the animal crawls away on its knees becomes progressively weaker and finally dies but the severity of the symptoms varies greatly in different individuals Very mildly affected animals apparently completely recover on transfer to "sound" pastures but where the symptoms are as just described no known treatment will effect recovery Pathologically the disease is characterized by degenerative changes (demyelination) in nerve fibers of the spinal cord and of the medulla oblongata and by hemosiderosis of the kidneys (110a)

Phalaris staggers was first described by McDonald (110a) who was led to suggest from a consideration of the nature of the condition and its restricted occurrence that it arises as a result of a soil deficiency which affects the metabolism of Phalaris so that it produces either an abnormal toxic substance or excessive amounts of some normal potentially toxic substance Support for this hypothesis came from later experiments by this worker in which topdressing of Phalaris pastures with a mineral *mixture consisting of salts of zinc cobalt boron manganese* copper and molybdenum were found to prevent the incidence of the disease (110a) It was shown further that staggers developed in sheep restricted to similar pastures topdressed singly with boron copper or

host animal but may be necessary for normal microbial function in the rumen

A satisfactory explanation of the relatively high requirement of the ruminant for *vitamin B_{12} must also wait further experimentation* In this connection it is *interesting to note that the* average vitamin B_{12} concentration of the liver of healthy adult ruminants is approximately twice that *of* similar nonruminant species (136) The intermediary metabolism *of ruminants* is known to differ markedly from that of nonruminants *particularly in respect to the* means by which they derive their energy *requirements* The main sources of energy to ruminants are the lower *fatty acids* (acetic propionic, and butyric) obtained by fermentation of carbohydrates in the rumen It can be postulated that the metabolism of large quantities of these substances imposes an especially high vitamin B_{12} requirement but for the present, this must remain as an attractive hypothesis only It should be appreciated also that although the curative requirements of vitamin B_{12} in the ruminant are large by comparison with those needed for the remission of pernicious anemia in man they are not so large when viewed in relation to the quantities available to the normal animal These are difficult to estimate because activities ranging from 50–60 μg vitamin B_{12} per 100 g of dry rumen contents by chick assay (68) to over 1000 μg per 100 g by *E coli* and *L leichmannii* assay (73a, 126) have been reported As was pointed out earlier the latter include a high proportion (up to 80–90%) of cobalt containing vitamin B_{12} like substances, other than vitamin B_{12} itself which appear to serve no useful purpose to the animal Furthermore even the true vitamin B_{12} concentration of the rumen ingesta of normal sheep can vary greatly under different dietary conditions (73a) If the average content of the dry ingesta of the sheep s rumen be taken as 800 g (8 liters × 10% dry matter) and 100 μg per 100 g of vitamin B_{12} is used as a "normal" concentration it can be calculated that the total true vitamin B_{12} present in the normal rumen is about 800 μg On this basis injections of 100–300 μg /week do not seem unduly large and the fact that the oral doses of 1000 μg /week given by Andrews and Anderson (6) were relatively ineffective suggests that vitamin B_{12} is very poorly absorbed in the ruminant This suggestion is supported by one study with vitamin B_{12} containing radioactive cobalt (123) and by a recent report indicating that less than 5% of the true vitamin B_{12} produced in the rumen is absorbed from the rumen of sheep (39) The reasons for this poor absorption are not yet known

It is not difficult to relate by analogy with human pernicious anemia the profound anemia of cobalt deficiency in ruminants to the shortage

at least in sheep Where staggers does not develop in animals grazing Phalaris pastures it is presumed that the soils are capable of maintaining sufficient concentrations of cobalt in the Phalaris or in the other components of the grazing to meet the extra requirements of this element imposed by the stress of the circumstances Whatever the mechanism ultimately shown to explain the incidence and control of Phalaris staggers however this disease remains as a remarkable example of soil plant animal interrelationship

III Cobalt in the Nutrition of Man and other Nonruminants

1 *Nonruminants other than Man*

Horses remain perfectly healthy when restricted to the grazings which induce symptoms of cobalt deficiency in sheep and cattle and all attempts to produce a state of cobalt deficiency in small laboratory animals have failed Rats have been shown to grow as well on semi synthetic diets supplying as little as 0 6 μg (150) or 0 3 μg cobalt per day (75) as when these diets were supplemented with additional cobalt and three generations of these animals have been raised successfully on diets which provided between 0 15 μg and 0 35μg cobalt daily according to the amount of diet consumed These rats revealed no obvious differences in growth and appearance from controls receiving additional cobalt (101) Rabbits have been maintained on diets supplying as little as 0 1 μg cobalt per day (144)

Since the concentrations of cobalt in these diets are much lower than those which will induce the cobalt deficient state in ruminants it can be deduced that their requirements for this element are extraordinarily low They must however have some need for dietary cobalt when consuming all plant rations because the higher plants contain little or no *vitamin B_1 and this vitamin is necessary for the animal's normal metabolic processes* The minimum cobalt requirement under these conditions would therefore be the amount needed by the intestinal flora to enable them to synthesize sufficient supplies of vitamin B_{12} for the host animal In herbivorous species such as the rabbit guinea pig and horse this situation applies at all times but in omnivores such as the rat and pig it applies only when the ration contains insufficient preformed vitamin B_1 The diets used in the experiments with rats described above would certainly supply appreciable quantities of vitamin B_1 and even in the experiments with rabbits the position would be complicated by their habit of coprophagy Whether nonruminant species require cobalt other than as a constituent of vitamin B_1 is unknown

manganese but not where zinc cobalt or molybdenum was applied (110a) The etiology of Phalaris staggers became much clearer when following a chance observation that a supplement of cobalt fully protected sheep from this demyelinating malady (102) Lee and Kuchel (92) obtained unequivocal evidence of its association with incipient cobalt deficiency and of its complete prevention by regular oral dosing of sheep grazing affected pastures with cobalt salts In 10 ewes which were dosed each week with 7 mg cobalt there was no incidence of staggers whereas 11 of 15 untreated ewes grazing with them were affected 6 of them fatally This important finding was subsequently confirmed with larger numbers of sheep (92)

Two alternative hypotheses have been advanced to explain the protective action of cobalt in Phalaris staggers (92) The first is that an increased cobalt concentration in the rumen contents favors the proliferation of particular microorganisms which have the capacity to destroy the neurotoxic principle in Phalaris before it is absorbed from the alimentary tract The second hypothesis is more interesting since it involves an analogy with a probable function of vitamin B_1 in human pernicious anemia In this condition vitamin B_{12} not only corrects the blood dyscrasia but also arrests the progress of the neurological defect (subacute combined degeneration of the cord) which is an almost invariable symptom of pernicious anemia and which seemingly occurs as a result of the accumulation of unidentified myelin destroying, neurotoxic substances arising from the same gastric defect which prevents the absorption of vitamin B_1 Since this neurological defect in man is overcome by vitamin B_1 it is argued that this vitamin functions as a detoxicating agent preventing the accumulation of harmful amounts of the neurotoxic substance In Phalaris staggers it is postulated that the neurotoxic principle ingested from the Phalaris which has not yet been isolated or identified similarly accumulates in sufficient concentrations to induce the demyelination responsible for the staggers syndrome under conditions of suboptimal cobalt and hence suboptimal vitamin B_1 status of the animals The provision of additional cobalt or the ingestion of sufficient cobalt from the ordinary diet, it is suggested prevents the disease by ensuring the production within the rumen of sufficient vitamin B_{12} to meet the normal metabolic requirements of this vitamin and in addition to detoxicate the neurotoxic substance

At this early stage in the investigation of the problem there is insufficient evidence to support fully either of these hypotheses or to provide any more acceptable explanation of the mode of action of cobalt in Phalaris staggers but there is no doubt of the protective action of cobalt

In the absence of any indication of a function for cobalt in man except in the form of vitamin B_{12}, the problem of cobalt in the nutrition of this species is one of the sources and supplies of this vitamin rather than of cobalt itself. All ordinary diets supply much more total cobalt than can be accounted for as vitamin B_{12}. The 5 μg of vitamin B_{12} per day which has been found adequate for the treatment of pernicious anemia when given orally with normal gastric juice contains only about 0.2 μg of cobalt. An average adult diet of good quality supplies 5–8 μg cobalt daily (69), the actual intake depending upon the nature of the diet consumed. Human foods vary considerably in cobalt content both within and between the different classes of foodstuffs (77). A compilation by Young (161) gives the cobalt content of plant materials consumed by man as varying from 1.2 p.p.m. of the edible portion of spinach to 0.005 p.p.m. of the edible portion of cherries. From the relatively few values available it appears that the green leafy vegetables, especially spinach, are the richest and also the most variable source of this element, and the cereal grains and the dairy products are the poorest source. Typical values taken from the data of Hurwitz and Beeson (77) are: spinach 0.4–0.5 p.p.m. cobalt on the dry basis, cabbage and lettuce 0.2 p.p.m., cowpeas (seed) 0.1 p.p.m., and corn (maize) 0.01 p.p.m. The concentration of cobalt in the dry matter of cow's milk is lower still, probably very close to 0.004–0.006 p.p.m. It can, however, as is shown later, be greatly increased by supplementing the diet of the cow with cobalt. There is no doubt also that the cobalt content of vegetables, especially the leafy vegetables, could be greatly increased by cobalt fertilization. There appears to be no justification for such treatment on any known soils because there is no evidence either of a need for a greater intake of cobalt than that normally obtained from ordinary human dietaries or of a stimulation of plant growth from the added cobalt. In fact, cobalt does not appear to be an essential element for the growth of plants.

IV. Cobalt Content of Animal Tissues and Fluids

1. *General Distribution*

Reliable data for the normal range of concentration of cobalt in body tissues and fluids are available for relatively few species, but they are sufficient to indicate that this element is widely distributed throughout the body in extremely low concentrations. There have been numerous observations of the distribution of radioactive cobalt in the tissues of mice, rats, rabbits, pigs, sheep, and cattle following oral or parenteral

Indications that the diets of simple stomached animals may not always contain sufficient cobalt to enable their intestinal organisms to synthesize adequate supplies of vitamin B_{12} for optimum growth have been obtained from several experiments with pigs (46) Additions of small amounts of cobalt (approximately 2 mg/kg feed) as cobalt chloride or cobalt carbonate to all plant rations (corn soybean oil meal) were shown by Dinusson and co workers (46) to increase the rate of gain and the efficiency of feed use of growing fattening pigs to a very small but significant ($P < 0.05$) extent In some experiments these workers obtained similar improvements over the basal ration by the addition of vitamin B_{12} or meat scraps but a response from cobalt supplements was observed with these rations even when they included 5% of meat scraps (46) These suggestive findings are of sufficient interest and importance to warrant further investigation of the value of cobalt supplements for growing pigs under rigidly controlled conditions in which the dietary intakes of both cobalt and vitamin B_{12} are critically assessed

2 *Man*

In the human species interest in cobalt as distinct from vitamin B_{12} has been focused very largely on its value as a nonspecific erythropoietic stimulant in the treatment of various types of anemia This function of cobalt, to be discussed later in relation to cobalt polycythemia has found some application in human medicine An earlier suggestion that this element might play some part in the normal treatment of the secondary anemias of man because of its invariable presence as a contaminant of iron salts and compounds (149) has received little support (18), but Kato (83) reports an accelerated formation of red cells and hemoglobin in infants suffering from nutritional anemia when iron treatment was supplemented with relatively large (50 mg) doses of cobalt Good results in post hemorrhagic anemias and anemias due to infections or tumors from similar doses of cobalt have been claimed (160) and significant erythropoietic responses have been obtained by treating the anemia associated with chronic renal disease with oral doses of cobalt chloride at the rate of 20 mg/day provided that the treatment is continued for at least a month (62) These latter results are of some practical importance because no therapy, other than transfusions or reduction of the uremia was previously known for this condition *

* Thyroid hyperplasia and hypofunction have very recently been reported by Kriss *et al* (89a) as serious toxic manifestations of prolonged cobalt therapy in children and adults suffering from anemia These authors warn against the indiscriminate use of cobalt especially in infants and children

There is some evidence of a similar placental transfer of cobalt in the sheep

Cobalt values for human tissues obtained by modern reliable methods of analysis are exceedingly scanty Heyrovsky (73) reports the following concentrations in two human cadavers expressed as p p m of cobalt in the fresh tissues liver 0 084 and 0 148 kidney 0 113 and 0 084 myocardium 0 018 and 0 015 brain 0 021 and 0 014 and lung 0 009 and 0 011 Conversion of these figures to the dry basis reveals that the concentrations do not differ greatly from those recorded for similar tissues in other species

2 *Cobalt in the Liver*

There is an extensive literature on the cobalt content of the liver particularly for sheep and cattle because liver cobalt determinations have been found extremely valuable but not infallible aids in diagnosing cobalt deficiency in the field The concentration of cobalt in the liver of these species varies very little with the age of the animal but it is markedly influenced by the level of cobalt intake in the diet This is evident from the figures for liver appearing in Table 20 and is supported by a considerable body of evidence from a wide range of environments Thus Underwood and Harvey (152) found the mean concentration of cobalt in the livers of a group of sheep suffering from cobalt deficiency to be 0 06 p p m on the dry basis compared with 0 28 p p m for healthy sheep and Marston and co workers (107) reported a mean of 0 09 p p m cobalt for the livers of coasty sheep compared with 0 34 p p m for a similar group of healthy cobalt treated animals Very similar values to these have been obtained for normal cattle (63 113) and for cobalt deficient cattle (113) From an extensive study of bush sick animals in New Zealand McNaught (113) suggests that 0 04–0 06 p p m or less in the dry matter of the livers of sheep and cattle indicate cobalt deficiency and that 0 08–0 12 p p m or more indicate a satisfactory cobalt status This worker showed further that unlike iron and copper cobalt does not normally accumulate in the fetal liver The cobalt concentration in the liver of the newborn lamb and calf is however reduced below normal when the mother has been on a cobalt deficient diet and can be raised above normal by prepartum cobalt feeding In both sheep and cattle under conditions of steady adequate cobalt intake it appears that the cobalt concentration in the liver rises very slightly from birth to weaning and is normally somewhat higher in the mature than in the newborn animal

The liver is frequently referred to as the storage organ of the body for

dosing with radioactive cobalt (26 37 130) All of these have shown that the retained cobalt is taken up in traces by all tissues with the highest concentrations occurring in the liver and kidneys and to a lesser extent in the pancreas Values obtained by conventional chemical methods similarly show that the highest concentrations of cobalt generally occur in all species examined in the liver, kidney and pancreas Occasional high concentrations have been reported also in the heart, spleen skin and cartilage The muscles are among the lowest of the tissues in cobalt concentration but because of their large mass they rival the liver as a site of total storage Nothing is known of the forms in which cobalt exists, or is bound in the tissues other than as a part of vitamin B_{12} In the ruminant there is no doubt that a very high proportion of the cobalt in some tissues notably the liver must normally exist in the form of vitamin B_{12} This point is considered further in the following section

The actual concentrations of cobalt in the tissues are greatly dependent upon the level of cobalt intake by the animal They are reduced below normal by subnormal intakes (14) and can be increased beyond normal levels by cobalt supplementation of ordinary diets (157) The effect of subnormal intakes of cobalt on the cobalt content of the tissues of young sheep is illustrated in Table 20 Ward and co workers (157) reported concentrations of cobalt in the tissues of normal newborn

TABLE 20

COBALT CONTENT[a] OF THE TISSUES OF COBALT DEFICIENT AND HEALTHY COBALT TREATED SHEEP (14)

Condition	Liver	Spleen	Kidneys	Heart	Pancreas	Gall Bladder	Blood
Cobalt deficient	0.02	0.03	0.05	0.01	0.02	0.02	0.01
Healthy cobalt treated *for 5 months*	0.15	0.09	0.25	0.06	0.11	0.03	0.03

[a] Measured in p p m Co on the dry basis

calves ranging from 0.10 to 0.22 p p m on the dry basis except for the skin and hair which contained the exceptionally high level of 1.0 p p m Newborn calves from cows which had received a dietary supplement of cobalt for 21-120 days prior to calving were found to contain substantially higher levels of cobalt in their tissues especially in the liver and kidneys where the increases were of the order of 50% These findings indicate that cobalt readily passes the placental barrier in the bovine

the ewe On the other hand there is as yet no evidence that the apparently lower vitamin B_1 potency of milk from ewes on unsupplemented normal rations imposes any limitation upon the growth or well being of the suckling lamb

V Cobalt Absorption and Excretion

Numerous studies with stable and with radioactive cobalt carried out with a wide range of animal species have shown that this element is very poorly retained by the tissues and whether administered orally or parenterally is almost completely eliminated from the body within a few days The pathways of excretion are the feces the bile and the urine but the proportion of the administered cobalt eliminated by these routes varies with the method of administration A high proportion of orally administered cobalt usually appears in the feces Fecal cobalt consists very largely of the unabsorbed fraction of the dose plus a small but significant amount which has been absorbed and excreted into the intestine via the bile Absorbed or injected cobalt by contrast is excreted very largely in the urine with a small fraction eliminated in the bile to form part of the fecal cobalt Cobalt is apparently not excreted into the intestine via the pancreatic juice (116) as might perhaps be expected from the relatively high concentration of this element consistently found in the pancreas

Such a pattern of cobalt absorption and excretion has been demonstrated in rats (65) rabbits (137) guinea pigs (110) cows (38) sheep (13 102) and man (87) Thus Greenberg *et al* (65) injected a 100 μg dose of radioactive cobalt into rats with biliary fistulae and found an average of 63 5% of this dose to be eliminated by way of the urine 5% in the feces and 3 5% in the bile within 72 hours After oral administration of a similar dose of radioactive cobalt excretion took place at the same rate and by the same pathways but 40% appeared in the feces 2% in the bile and 18 5% in the urine Comar and co workers (38) injected 174 μg of radioactive cobalt into the jugular vein of a rumen fistula cow and recovered about 65% of this dose in the urine about 7% in the feces and 6% in the blood within several hours Very small amounts were detected in the saliva but none in the rumen When these workers introduced the same amount of radioactive cobalt directly into the rumen 82% was found to be still in the rumen after 11 hours After 7 days this had decreased to 1% of the original dose by which time over 65% was accounted for in the feces and only very small amounts in the urine Askew and Josland (13) drenched sheep with a single 4 mg dose of cobalt as cobalt chloride and followed the course and rate of excre-

Archibald (9) 0.2–1.1 (mean 0.6) $\mu g/l$ The average cobalt content of cows colostrum is almost 10 times higher namely 5 $\mu g/l$ (98)

Whether under conditions of cobalt deficiency, the concentration of cobalt in milk is reduced below normal, as occurs with copper under conditions of copper deficiency has not yet been determined but the concentration in colostrum can readily be raised above normal by pre partum cobalt supplementation of the cow (157) and in milk during full lactation by supplementing normal rations with liberal amounts of cobalt Archibald (9), using a double reversal method with 4 cows found that a supplement of 120 mg of cobalt as cobaltous acetate daily for 4 months increased the cobalt content of the milk four fold, that is from a mean of 0.6 to a mean of 2.4 $\mu g/l$ It is apparent that in contrast to the position with iron and copper, the mammary gland readily permits cobalt to pass its barriers and that such treatment represents an easy practical means of raising the cobalt intake of suckling calves in cobalt deficient areas

The question of the proportion of the cobalt in milk which normally occurs as vitamin B_{12} cannot be answered with any confidence because the values reported for the concentration of this vitamin by different investigators vary greatly and cobalt and vitamin B_{12} assays have, so far as is known not been made on the same samples However several groups of workers have obtained values for cow s milk averaging between 6 and 8 μg of vitamin B_{12} per liter (8 36 71) On the basis of 4% cobalt in vitamin B_{12} this represents a concentration of 0.24–0.32 μg cobalt per liter or about half the total cobalt found to be present in normal cow s milk in other investigations Supplementing the normal ration of cows with additional cobalt is ineffective in raising the vitamin B_{12} activity of the milk (71) although as mentioned earlier such supplementation is highly effective in raising the total cobalt content of the milk Dietary supplements of cobalt to rations already containing at least the minimum requirements of this element have by contrast been shown to produce significant increases in the vitamin B_{12} activity of ewes milk (70 115) The effect is especially marked in the colostrum and during the first few days of lactation

The significance of these findings in the sheep and the reason for the absence of any such response to cobalt supplements to normal rations in the dairy cow can only be established by further experimentation under controlled conditions but the results obtained so far suggest that the minimum intakes of cobalt established as necessary for normal health growth and reproduction in the sheep are inadequate for maximum vitamin B_{12} synthesis and transfer from the blood to the milk of

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when cobalt administration is continued for some time and still larger doses than those required for the polycythemic effect may depress erythropoiesis and actually cause anemia (20 60)

The stimulating effect of cobalt on erythropoiesis can also be depressed or enhanced by various means which do not appear to act in the same way in all species Thus in the dog the simultaneous or separate feeding of liver and choline or the injection of liver extracts or ascorbic acid either reduces or nullifies the effect of cobalt (42) No such depressing effect of choline has been found in the rat (and by some investigators in the dog) and whole liver powder liver extract or a liver concentrate far from depressing actually accentuated cobalt polycythemia in rats on a mineralized milk diet (3) The mode of action *of these substances is little understood but the manner in which certain* other compounds function in the inhibition of the polycythemic action of cobalt seems clearer Thus ethylenediamine tetracetic acid (EDTA) which forms stable nonionic chelate compounds with metals including cobalt prevents the development of polycythemia when fed with cobalt to rats presumably by combining with the cobalt to form a nonionized compound from which the cobalt cannot be absorbed (33) Concurrent injections of methionine cystine cysteine or histidine in rats and calves reduce the toxicity of high levels of orally ingested cobalt apparently as a result of their capacity to form coordination complexes with this element which are relatively inactive (66) Several workers have shown that cobalt forms coordination complexes with certain amino acids (30) and the complex of cobalt and cysteine formed *in vitro* is relatively nontoxic in the rat (114)

The potency of cobalt as an erythropoietic agent has attracted considerable attention largely because of the hope that an understanding of its mode of action would provide some clue to the normal regulatory mechanism of erythropoiesis Many theories have been advanced and a great deal has been learned of the ways in which cobalt does *not* act but the underlying mechanism of cobalt polycythemia remains obscure (64a) Cobalt does not induce a passive accumulation of erythrocytes does not affect the nature of the hemoglobin or its oxygen carrying capacity and does not produce a cobalt containing methemoglobin (28) Studies with radioactive iron indicate that cobalt does not increase the utilization of iron and injections of large doses of vitamin B_1 do not significantly increase hematopoiesis in the rat suggesting that this element does not induce polycythemia by way of intermediary formation of vitamin B_1 (94) The suggestion of Barron and Barron (17) that cobalt inhibits the respiration of immature red cells causing their dis

tion After 5 days virtually the whole of this dose was accounted for in the urine and feces, indicating very little retention in the tissues Only 2% of the cobalt in the drench appeared in the urine, the remainder other than the minute amount stored in the body, was excreted in the feces The rapidity with which these animals excrete cobalt was shown by the fact that a high proportion of the dose to be excreted in the feces appeared in the first 48 hours and a large proportion of the small amount to be excreted in the urine appeared in the first 24 hours after drenching

Evidence that this pattern of excretion of ingested cobalt is not necessarily followed in man, at least when this element forms a part of ordinary diets at very small intakes is presented by Harp and Scoular (69) These investigators conducted cobalt balance experiments on 23 young women consuming high quality self selected diets With such diets which provided an average daily intake of 6 7 μg cobalt, 73-97% of the dietary cobalt was absorbed and an average of 67% appeared in the urine The daily fecal excretion in 20 of the 23 subjects was less than 1 μg cobalt

VI Cobalt Polycythemia and Cobalt Toxicity

1 *Polycythemia*

Since the original observation of Waltner and Waltner (156) that polycythemia can be induced in rats by the oral or parenteral administration of cobalt in doses large by comparison with the amounts ordinarily consumed in the food many investigators have confirmed this phenomenon in rats and showed that it occurs also in mice rabbits guinea pigs dogs, pigs frogs, ducks chickens and man (64a) Polycythemia is not induced in mature ruminants by feeding or injecting equivalent amounts of cobalt per unit of body weight (20 64 78), although typical increases in hemoglobin level erythrocyte count and packed cell volume have been demonstrated in calves as among the first effects of excessive cobalt consumption (86)

Cobalt polycythemia only develops when the diet is otherwise adequate for rapid blood formation and has been shown not to occur when the diet is deficient for instance in iron or copper It is a true polycythemia accompanied by augmentation of the blood volume hyperplasia of the bone marrow and an initial or a maintained reticulocytosis The red cells contain hemoglobin which is apparently no different from that ordinarily formed and they survive for the normal length of time when transfused from a cobalt treated person into another individual (22) The degree of polycythemia frequently shows some fluctuations

receiving more than 0 6 mg cobalt per day whereas 1 0 and 1 5 mg /day induced a rapid loss of weight and 2 0 mg /day caused death in two weeks Several workers have shown that about 200 p p m of cobalt in the ration of rats produces toxic effects resulting in lowered food consumption and decreased growth and that 500 p p m is lethal within a few weeks (79) The toxic level per unit of body weight is considerably less than this in calves Variation in tolerance between individuals is great but it appears that up to approximately 40 mg cobalt per 100 lb body weight daily is tolerated by most bovines without observable detrimental effects (49 86) Intakes of cobalt in excess of this level usually result in a depression of appetite and loss of weight The physiological condition of the animal is an important factor in determining the toxic level For instance detrimental effects have been reported in lactating dairy cows with single doses of 1–5 g cobalt sulfate for 3–4 months before showing extreme emaciation (49) Sheep are highly tolerant to excessive oral intakes of cobalt Becker and Smith (20) drenched yearling sheep daily for 8 weeks with cobalt chloride at levels ranging from 10 to 500 mg of elemental cobalt per 100 lb body weight Levels up to 160 mg daily were found to be tolerated by these animals without harmful effects but dosages of 200 or 500 mg cobalt per centweight daily caused a severely depressed appetite and body weight losses with an anemia at the higher level There was no evidence of polycythemia at any dose level Intakes of this order are many hundreds of times greater than the cobalt requirements of these species It is obvious that the practical possibility of overdosage sufficient to cause cobalt toxicosis is exceedingly remote

VII Vitamin B_{12}

Full consideration of all aspects of vitamin B_{12} lies clearly outside the scope of a text on the trace elements Since however the only known function of cobalt within the tissues of animals is as a component of the vitamin B_{12} molecule and the metabolic roles of the two nutrients cannot at present be dissociated effective consideration of the metabolic significance of cobalt necessitates the devotion of some attention to relevant aspects of vitamin B_{12} With this end in view a brief account of the nature potency occurrence and functions of vitamin B_{12} and similar cobalt containing compounds is presented below The relation of this vitamin to cobalt deficiency in ruminants has already been discussed and is therefore only incidentally referred to in this section For a very thorough and extensive review of most aspects of vitamin B_{12} the reader is referred to a recent text (134)

charge into the circulation as imperfect, nonrespiring cells and their replacement in the bone marrow by new cells, has not been confirmed (158) No vascular changes can be seen in the bone marrow of animals with cobalt polycythemia (158)—only marked erythropoietic activity—and the experiments of Warren and co workers (158) exclude any effect mediated by way of the peripheral nervous system

The most attractive hypothesis is that the effects of cobalt are due primarily to the formation of cobalt coordination complexes with compounds in the cell such as cysteine or glutathione, thus fixing thiol (—SH) groups and interfering with cellular respiration within the blood forming organs and in this way providing the necessary stimulus to erythropoiesis (66) Cobalt is known to inhibit the respiration of certain bacteria perhaps by the formation of partially reversible complexes with cysteine and histidine (29) and the administration of cysteine in amounts sufficient to form a stable association complex with cobalt lessens materially the erythropoietic effect of cobalt (119)

No completely satisfactory explanation of the mechanism of cobalt polycythemia has yet been produced The failure of cobalt to induce polycythemia in mature ruminants is unexplained and no worth while clues to the regulatory mechanism of normal erythropoiesis have emerged from the studies of cobalt polycythemia *It is clear nevertheless* that cobalt is a nonspecific erythropoietic stimulant which is effective under a remarkably wide range of conditions The polycythemia of cobalt *can be summated with the polycythemia of altitude* (35) and with the marrow stimulation which follows hemorrhage (88) whilst the anemia which follows hypophysectomy in mature female rats can be completely prevented by cobalt administration in appropriate doses (41) Its effectiveness in the treatment of the anemia associated with renal disease and other dysfunctions has already been mentioned

2 *Cobalt Toxicity*

The widespread use of cobalt salts in the prophylaxis and therapy of cobalt deficiency in sheep and cattle has stimulated interest in the tolerance level of animals for this element Appreciable differences among species and wide variations among individuals within species in tolerance to relatively high doses of cobalt exist but in general cobalt salts are not particularly toxic to animals A very wide margin of safety exists between the quantities of cobalt necessary to fulfill the nutritional requirements of sheep and cattle or likely to be used in their treatment *and the toxic limits* of this element in these species

Stare and Elvehjem (139a) observed toxic effects with young rats

Of the greatest importance is the fact that these vitamin B_{12}-like substances differ markedly in their capacity to promote the proliferation of various test organisms and to function in the higher animals in the same way as cyanocobalamin or its analogues mentioned in the previous paragraph. Pseudovitamin B_{12} has a high vitamin B_{12} activity for certain lactobacilli but cannot replace this vitamin in the nutrition of rats, chicks, or man (124); vitamin B_{12f} promotes the growth of *L. leichmannii*, *E. coli*, and *Euglena gracilis* but cannot fulfill the vitamin B_{12} requirements of rats or chicks (95), and cyano w cobalamin and fraction B are inactive when given in the diet of chicks, although the former has been found clinically active in pernicious anemia (59). Thus many cobalt-containing complexes are formed by microorganisms which are similar in many respects to the cobalamins but which may differ sufficiently from vitamin B_{12} to render them useless, or at best inefficient, as a source of this vitamin to the higher animals.

2. *The Occurrence of Vitamin B_{12}*

The key to the distribution of vitamin B_{12} in natural materials is that it originates only in microorganisms. It is not produced by the higher plants and occurs in animal tissues solely as a result of the ingestion, directly or indirectly, of the products of microbial fermentation. A great many bacteria and molds produce vitamin B_{12}, but not all microorganisms have this capacity. The yeasts, for example, apparently neither require nor synthesize vitamin B_{12}. Other species of microorganisms, including those employed in assays of this vitamin, require it but cannot produce it for themselves. Among this latter group are types of microorganisms such as the chrysomonad *Ochromonas malhamensis*, which must be supplied with vitamin B_{12} itself, whereas others are less exacting and can effectively utilize a number of B_{12}-like substances and even thymidine and certain deoxyribosides. The chlorophyll-bearing flagellate *Euglena gracilis* cannot utilize thymidine or deoxyribosides and is particularly sensitive to deprival of vitamin B_{12} from its growth medium (76). By contrast, the blue-green algae, since they have been shown to contain substantial quantities of vitamin B_{12} (128), apparently readily produce this vitamin. The relatively large amounts of vitamin B_{12} that accumulate in mollusks such as oysters and clams and in bony fish may in fact be derived from this source (134).

Assessment of the vitamin B_{12} content of human and animal foods is fraught with many difficulties. The results obtained by microbiological assay will clearly depend upon the test organism employed. Vitamin B_{12} activity measured by *E. coli* or *L. leichmannii* assay may include a

I The Nature and Potency of Vitamin B_{12} and Related Compounds

A great deal has been learned of the nature of the vitamin B_{12} molecule since its isolation from mammalian liver, in 1948 as a red crystalline compound containing 4.0 per cent cobalt (137a). This compound has very recently been shown to have the empirical formula $C_{63}H_{90}O_{14}N_{14}PCo$ and a complete molecular structure for the vitamin has been advanced based upon X-ray studies and considerations of its chemical behavior (64). The cobalt moiety is known to occur as a cyanocobalt coordination complex in which the coordinatively bound cyano group is replaceable by other ions or molecules (27). The demonstration of the nature of the cobalt complex in vitamin B_{12} was of the greatest importance since it enabled a system of nomenclature to be devised which has been applied to the various modifications of this vitamin present in many natural materials.

These substances were given the somewhat confusing names of vitamin B_{12a}, B_{12b}, B_{12c}, B_{12d}, and so on. Under this system of nomenclature the name *cobalamin* designates all the vitamin B_{12} molecule except the cyano group. Vitamin B_{12} is then cyanocobalamin, vitamin B_{12a} is hydroxocobalamin, and vitamin B_{12c} is nitritocobalamin. Vitamins B_{12b} and B_{12d} have been shown to be identical with vitamin B_{12a}. All these compounds originate in nature as products of the metabolic activity of various microorganisms and are readily interconvertible. They show essentially similar activity when assayed by different methods and can be regarded as physiologically equivalent in supplying the nutritional requirements of both higher animals and appropriate microorganisms. Thus vitamin B_{12} and B_{12a} are equally effective in promoting the growth of *L. lactis* and *L. leichmannii* and of rats and in the treatment of pernicious anemia in man, whereas the chick assay method indicates an activity for vitamin B_{12a} approximately 50% of the activity of vitamin B_{12}. Similarly, as pointed out earlier, vitamins B_{12} and B_{12b} are equally effective in the treatment of cobalt deficiency in lambs.

Several other vitamin B_{12}-like substances occur along with vitamin B_{12} in the rumen, abomasum, and intestines and in the feces of animals and birds. Various names such as pseudo-vitamin B_{12} (124), cyano-w-cobalamin (34), factors B and C (34), and vitamin B_{12f} (95) have been given to these substances by different groups of workers and some of them have been prepared in crystalline form. They are known to differ in important respects from the cobalamins but much remains to be learned of their chemical nature. Even some of the crystalline materials have been separated into several components by ionophoresis (57). Detailed consideration of their nature will therefore not be attempted.

buffalo sheep and goat) all fell within the range 1 18–1 33 μg/g liver tissue The values for pig and rabbit livers were approximately half of the average value for beef liver and that for chicken liver was nearly one fourth In confirmation of the work of earlier investigators the vitamin B_{12} content of rat liver was found to be the lowest being less than a twentieth of that of beef liver

The main normal dietary sources of vitamin B_{12} to farm animals are the animal protein concentrates especially fish meal fish solubles, liver meal and meat meal Milk buttermilk and whey may also be effective sources The use of suitable fermentation materials however in which vitamin B_{12} has been produced by microbial growth has placed a relatively cheap and plentiful source of this vitamin in the hands of animal feeders so that supplies of vitamin B_{12} for pigs and poultry subsisting on all vegetable diets can readily be obtained without recourse to products of animal origin This question is taken up further in the section dealing with the "animal protein factor"

The vitamin B_{12} activity of animal tissues determined by *L leichmannii* or similar assay is due very largely to cyanocobalamin itself or its equally potent analogues (The pancreas appears exceptional in that a high proportion of the B_{12} activity by *L leichmannii* assay is due to the presence of deoxyribosides (132)) This is not necessarily true of all materials used as a source of vitamin B_{12} for the higher animals—a matter of considerable importance in view of the low potency or non potency of certain vitamin B_{12} like substances in the nutrition of these animals This has been strikingly and convincingly demonstrated by Hine and Dawbarn (73a) who recently determined the vitamin B_{12} activity of the rumen contents of sheep by four microbiological methods namely *E coli* mutant plate assay and *E coli* mutant *L leichmannii* and *Ochromonas* tube assays The fourth test organism was a chrysomonad *Ochromonas malhamensis* which had been shown by Ford (56) to be specific for cyanocobalamin itself and not to respond to pseudo vitamin B_{12} cyano w-cobalamin or Factors B and C Hine and Dawbarn found that in the presence of adequate dietary cobalt the vitamin B_{12} activity of the rumen contents as determined by *E coli* assay was 10 to 20 times the true vitamin B_{12} activity as measured by the *Ochromonas* assay Withdrawal of the supplementary cobalt from sheep subsisting on a cobalt deficient ration was followed by a spectacular fall in the B_{12} activity of the rumen contents as determined by all four assay procedures Further the ratio of *E coli* activity to *Ochromonas* activity also fell but much less markedly so that the ratio was reduced to less than 10 and remained below 10 (Table 21) The

number of substances which cannot be used by the higher animals as a source of vitamin B_{12}. This will depend upon the materials under test. Fortunately, animal tissues which constitute the main dietary source of this vitamin to animals and birds contain little or no vitamin B_{12}-like compounds other than B_{12} itself (43), but assays of intestinal sources may give completely misleading results unless the right microorganism is used. This point is referred to in more detail later. The extraction procedures used in the assay can also affect the results obtained. Even direct assay of the vitamin B_{12} content of different materials on pernicious anemia patients may be complicated or invalidated, because of the presence of folic acid, especially as folic acid is more readily utilized by the oral route in this disease than is vitamin B_{12}.

Many of the early assay difficulties and discrepancies have now been resolved so that a reasonably clear picture of the distribution of vitamin B_{12} in human and animal foods can be presented. All foods of higher plant origin, such as cereals, grasses, legumes and their seeds, roots, tubers and leafy vegetables, contain no vitamin B_{12}, or negligible concentrations. The same is true of yeast. All animal tissues and fluids, on the other hand, contain some vitamin B_{12}, although the amounts vary greatly with the tissue and with the species. Among human foods, mammalian (especially ruminant) liver and kidney and shellfish are the richest sources, followed by seafish, muscle meats and milk. A recent classification (134) divides human foods as sources of vitamin B_{12} into three groups as follows:

Excellent Sources (0.5 μg or more, per gram of dry matter). Mammalian liver and kidney, oysters, clams.

Good Sources (0.05–0.5 μg per gram of dry matter). Lean beef, lamb, veal, poultry meat, salt water fish, milk.

Poor Sources (in many cases giving no assay response). Cereal grains, leguminous seeds, green leaves, vegetables, yeast.

The higher vitamin B_{12} potency of organ meats than of muscle meats has been demonstrated by several groups of workers. Scheid and Schweigert (132), for example, examined composite samples of liver, kidney, pancreas, heart, spleen, and lung, and samples of muscle meats from beef, pork, and lamb carcases. Liver and kidney were found to be the richest sources and the meats of beef organs and, to a lesser extent, lamb organs were shown to be higher in vitamin B_{12} potency than those of pork. The potency of spleen, heart, brain, and lung was slightly higher than that of beef and pork muscle tissues. In a more detailed study of animal livers from different species, Shenoy and Ramasarma (136) found that the vitamin B_{12} activity of livers from ruminants (beef

TABLE 21

VITAMIN B_{12} ACTIVITY OF RUMEN CONTENTS OF A SHEEP GIVEN 1 MG Co *per os* PER DAY FOR 14 DAYS THEN Co WITHHELD[a] (73a)

Sample number[c]	μg B_{12} activity per g dry rumen contents[b]: *E coli* plate (a)	*E coli* tube (b)	*L leich mannii* (c)	*Ochromonas* (d)	Ratio (a/d)	(b/d)	(c/d)	(d/d)	% dry wt	Food intake (g /day)	Cobalt in fodder (p p m)	Time after final administration of cobalt
B 1	11 7	2 2	1 57	0 84	14	2 6	1 9	1	8 1	875	0 012	2 hours
B 2	12 1	2 3	1 57	0 91	13	2 4	1 7	1	7 9	843	0 012	24 hours
B 3	4 3	1 21	1 15	0 75	5 7	1 6	1 5	1	7 5	884	0 012	48 hours
B 4	1 65	0 54	0 47	0 34	4 8	1 6	1 4	1	8 5	680	0 012	68 hours
B 5	1 03	0 43	0 34	0 187	5 5	2 3	1 8	1	6 8	496	0 012	92 hours
B 6	0 59	0 22	0 139	0 074	8 0	2 9	1 9	1	7 4	520	0 012	5 days
B 7	0 50	0 165	0 101	0 068	7 4	2 4	1 5	1	7 8	657	0 012	6 days
B 8	0 47	0 171	0 095	0 070	6 8	2 4	1 4	1	7 6	685	0 012	7 days
B 9	0 36	0 153	0 081	0 067	5 4	2 3	1 2	1	9 4	919	0 012	9 days
B10	0 51	0 163	0 102	0 083	6 1	2 0	1 2	1	9 5	898	0 012	12 days
B22	0 52	0 190	0 123	0 088	6 1	2 2	1 4	1	8 3	987	0 012	112 days
B23	0 81	0 29	0 180	0 100	8 1	2 9	1 8	1	7 8	758	0 016	133 days

[a] Diet wheaten hay chaff containing 5% gluten *ad lib*

[b] Rumen contents obtained through a fistula

[c] Samples collected immediately prior to feeding except B1 B2 and B3 which were collected 1 to 5 hours after feeding

rumen contents of sheep taken directly from normal pasture revealed in general both a higher total activity and a higher proportion of true vitamin B_{12} than the cobalt supplemented pen fed animals cited above but a considerable proportion of the total activity was still due to the presence of substances with no known value to the animal

3 *Vitamin B_{12} and the Animal Protein Factor*

It is apparent from the nature of the occurrence of vitamin B_{12} outlined above that animals subsisting on ordinary all plant or all vegetable diets will ingest little or none of this vitamin Such animals must therefore be dependent upon the synthetic activities of their intestinal micro flora Ruminants with their vast numbers of ruminal microorganisms, readily obtain their vitamin B_{12} requirements in this way, except as described earlier when their diet is deficient in cobalt The rarity of clinical evidence of a deficiency in man coupled with the essential limitation of dietary sources of vitamin B_{12} to foods of animal origin suggest strongly that a substantial proportion of human B_{12} requirements is supplied by intestinal bacterial synthesis Simple stomached farm animals such as pigs and poultry may obtain additional supplies from their environment through their habit of coprophagy or the consumption of litter and refuse in which bacterial fermentation with organisms voided in the feces or contaminated from the soil has taken place Where these species are fed all vegetable rations under conditions which eliminate this latter source of vitamin B_{12} the intestinal supplies may be insufficient to meet their full nutritional requirements

For many years chicks and young pigs have been known to grow and thrive much better on rations containing animal protein concentrates than on rations supplying the same amount of protein from vegetable sources such as soy bean meal Various investigations soon established that the benefits of these animal protein sources were not due to the essential amino acids or the known vitamins which they supplied but to an unidentified dietary factor to which the name "animal protein factor" (APF) was applied Dried cow manure rumen contents and a crude concentrate from the dried mycelium of *Streptomyces aureofaciens* were also shown to be rich sources of APF Finally in 1948 crystalline vitamin B_{12} was found to have APF activity (120) Subsequent investigations in many laboratories revealed that the APF activity of many materials could be largely accounted for by their vitamin B_{12} content The marked growth stimulating effect of certain other materials notably mold preparations from the antibiotics industry was found to be due partly to their vitamin B_{12} content and partly to the antibiotic

present The history of these important advances and the whole question of the nutritional effects of antibiotics and their relation to vitamin B_{12} and the animal protein factor effect have been detailed elsewhere (82 163)

While these researches with pigs and poultry were proceeding a series of somewhat similar investigations was being independently undertaken with the rat In the early 1930s a deficiency condition during pregnancy and lactation was demonstrated in this species which could be prevented or overcome by supplementing the particular all plant diets with fresh liver or liver extract Names such as "physin (99) and factor X" (31) were given to the unknown protective factor in liver Ultimately as with the animal protein factor the liver factor was identified as vitamin B_{12}

The outstanding manifestation of vitamin B_{12} deficiency in rats pigs and poultry is an impairment of the very early growth of the animal Anemia occurs but it is not a conspicuous feature of the B_{12} deficiency syndrome as it is in folic acid deficiency Nor do neurological symptoms develop as in pernicious anemia except to some extent in vitamin B_{12} deficient pigs Adult animals can subsist for long periods on diets comprising plant products only with little apparent effect on their weight or well being but the importance of vitamin B_{12} to the young of these species in their early growth stages can readily be demonstrated A high mortality has been observed in suckling rats from mothers fed a B_{12} deficient diet during pregnancy and lactation (133), and the hatchability of eggs laid by B_{12} deficient hens is invariably low due to death and failure of normal growth and development of many of the embryos (118) Even when chicks are successfully hatched from such eggs they grow poorly and mature slowly and the mortality is high during the first few days of life

4 *Vitamin B_{12} and Pernicious Anemia*

Immediately following the isolation of vitamin B_{12} from liver injections of the crystalline vitamin were found to produce hemopoietic remissions in pernicious anemia and an amelioration of the neurological symptoms and glossitis associated with this disease Unlike folic acid which gives a hemopoietic response only vitamin B_{12} can completely replace the potency of injectable liver preparations The effectiveness in pernicious anemia of concentrated liver extracts appears to depend entirely on their content of B_{12} (cyanocobalamin) and its analogues The amount of injected vitamin B_{12} necessary to produce and maintain a satisfactory clinical condition is only about 1–2 μg daily but it is very much less effective by mouth, unless normal gastric juice or some other source of

the intrinsic factor is given simultaneously (23). The oral effectiveness of vitamin B_{12} in pernicious anemia varies greatly in different patients due to the fact that some individuals with this disease still secrete small amounts of the intrinsic factor and hence are able to utilize oral vitamin B_{12} to some extent. It has been shown that a single oral dose as large as 3 mg. of vitamin B_{12} will generally secure a good remission of pernicious anemia in relapse (153). Oral treatment with large doses of vitamin B_{12} of this order may eventually come to be the routine treatment for pernicious anemia, especially with the improved methods and lowered costs of production of this vitamin.

The time between cessation of therapy and the onset of relapse in pernicious anemia is of considerable interest. Erf and Weiner (50) reported that the injection of 50–100 μg. of vitamin B_{12} produced a remission lasting from 50 to 100 days and that the administration of 50 μg. to a patient already in remission would prolong the remission for 70 to 120 days. More recently it has been shown that single massive injections of the order of 1000 μg. of vitamin B_{12} secure remission of pernicious anemia lasting from 120 to 365 days (155). Individual variability in the length of time a remission will last upon the withdrawal of therapy is very great, but the results just cited suggest that vitamin B_{12}, unlike most of the water-soluble vitamins, is normally stored in the tissues for considerable lengths of time. In ruminants the position appears to be different, as was pointed out earlier. A single massive injection of vitamin B_{12} into cobalt-deficient lambs produced only a brief response (6).

Clinical experiments with normal gastric juice and other duodenal and gastric mucosa preparations have established that vitamin B_{12} is identical with Castle's extrinsic factor and also apparently with the erythrocyte maturing factor of liver postulated by Castle as the product of interaction between intrinsic and extrinsic factors (32). There is no doubt also that the intrinsic factor, whose absence from the gastric juice of pernicious anemia patients represents the essential metabolic defect in this disease, functions by facilitating the absorption of B_{12} from the alimentary tract. How this is accomplished or whether an effect on absorption is the sole function of intrinsic factor remain to be determined. Some progress has been made towards the elucidation of the former problem by the demonstration of a binding action of gastric juice on B_{12}. The unstable complex so formed could protect the vitamin against utilization or inactivation by intestinal bacteria so that it becomes available to the host.

The therapeutic effect of B_{12} on the neurological lesions of pernicious anemia, which is not exerted by pteroylglutamic acid (PGA), indicates

that B_{12} is concerned with metabolic processes affecting the integrity of the neurones This function of B_{12} is not understood and its therapeutic role in the treatment of various neuropathies associated with a miscellaneous group of conditions other than pernicious anemia is also obscure It is a curious and unexplained fact that in folic acid deficiency in man nervous lesions are not at all striking whereas in animals such lesions may be marked in folic acid deficiency but not in vitamin B_{12} deficiency except, as was mentioned previously, in pigs The hemopoietic disturbances which represent the most common and clinically important manifestations of deficiency of both B_{12} and PGA, are evidently closely interrelated In fact it is widely held, as stated by Bethell (134) "that megalobastosis and other marrow changes characteristic of pernicious anemia and related conditions are the direct result of what may be termed PGA metabolic dysfunction This does not necessarily imply an actual deficiency of PGA, since the enzymatic function of this vitamin is dependent upon its presence in biologically active form as well as upon the completion of biologic reactions which require the participation of other substances such as B_{12} This question is considered further in the section dealing with the metabolic roles of vitamin B_{12}

The concept of pernicious anemia as a conditioned nutritional deficiency disease which arose as a result of the introduction of liver therapy and which was supported by later studies with pure vitamin B_{12} has tended to denigrate an earlier hypothesis which associated the disease with intestinal sepsis and the elaboration and absorption of hemolytic toxins Nevertheless persistent claims have been made that toxic substances are involved In pernicious anemia in relapse there is a high blood phenol level and an increased urinary excretion of phenolic compounds which is corrected by administration of vitamin B_{12} (142), and the blood serum of pernicious anemia patients unlike that of normal individuals inhibits the transformation of megaloblasts to normoblasts when added to cultures of bone marrow *in vitro* (131) Moreover rats with surgically induced intestinal stenosis have been shown to develop a macrocytic anemia which responds to aureomycin (145) and several groups of workers have obtained favorable responses in pernicious anemia to various antibiotics The action of antibiotics in pernicious anemia could be due to a reduction in intestinal organisms which deprive the host of this vitamin but it could also be due to the removal of bacteria that elaborate the postulated injurious substances within the intestine The further possibility has been suggested that antibiotics act by producing alterations in intestinal flora with the establishment of species that synthesize and release significant amounts of readily absorbable PGA or

related compounds (96) It must be emphasized however that whatever other nutritional factors or toxic agents may be operative in pernicious anemia they are completely counteracted by supplying vitamin B_{12} to the tissues

5 *Metabolic Roles of Vitamin B_{12}*

Vitamin B_{12} has been related to various metabolic processes in vertebrates These include the metabolism of proteins the synthesis of ribonucleic acid the synthesis and transfer of methyl groups, the utilization of one carbon fragments and the metabolism of tyrosine Vitamin B_{12} has been related also to liver function and thyrotoxicity in animals The precise role of the vitamin in these processes is little understood Certain of the more important of them are considered briefly below

a *Vitamin B_{12} and Protein Metabolism in Animals* Although the role of vitamin B_{12} in protein metabolism is at present ill defined a range of diverse evidence suggests that some relationship exists A deficiency of factor X (later identified with vitamin B_{12}) was found to be accentuated by raising the vegetable protein level in the diet of rats (31) abnormally high nonprotein nitrogen and urea levels have been demonstrated in the blood of B_{12} deficient rats (162) and chicks (111) which returned to normal levels with the administration of B_{12} acute uremia which responded to injections of B_{12} was observed in newborn rats from B_{12} deficient mothers (133) and the biological value of casein and nitrogen assimilation were shown to be significantly lower in B_{12} deficient than in normal rats (72) Further a protein sparing action for vitamin B_{12} has been proposed from studies of the effect of thyroxine in B_{12} deficiency Suggestive as they are these findings cannot yet be interpreted as providing proof of a direct and specific function for vitamin B_{12} in protein metabolism

b *The Synthesis and Transfer of Methyl Groups* The disclosure originally made by the work of du Vigneaud and his co workers (154) of the existence of a transmethylating process in animal tissues and of the consequent nutritional importance of biologically labile methyl groups was followed by a series of investigations which revealed the importance of vitamin B_{12} to both the synthesis and the transfer of methyl groups at least in rats and chicks Du Vigneaud *et al* found that under certain conditions homocysteine could not replace methionine in the diets of young rats unless choline was supplied to provide the methyl groups Subsequently several groups of workers showed in both rats and chicks that this requirement for methyl groups was reduced by supplying a source of vitamin B_{12} Conversely it was demonstrated that the require

ment for B_{12} by these species is reduced but not eliminated by ample supplies of methyl donors (81) When vitamin B_{12} is supplied, rats and chicks can evidently utilize certain amino acids as sources of the methyl group for methionine synthesis but in the absence of this vitamin a source of labile methyl' such as choline or betaine is required for methylation of homocystine It appears further, that even in the presence of ample B_{12}, a source of labile methyl groups is essential in the diet of very young animals whose rate of synthesis of these groups seems to be insufficient to meet the demands of rapid growth In the adult animal supplied with adequate B_{12}, the synthesis of methyl groups can probably proceed sufficiently rapidly to meet the requirements of maintenance

From studies with C^{14} it has been shown that the rat can form methyl groups from a number of substrates as long as an adequate supply of vitamin B_{12} or of pteroylglutamic acid is available to the animal These substrates include formate formaldehyde, acetone methyl alcohol the α carbon of glycine and the β carbon of L serine (see Porter 126) but little is yet known of the importance of any particular precursor as a source of methyl groups during normal metabolism or of the relative ease with which these groups may be formed from them It seems certain however that vitamin B_{12} functions in the synthesis of methyl groups from nonessential dietary constituents

c *Utilization of Single Carbon Fragments* The participation of such sources of single carbon fragments as formic acid formaldehyde and methyl alcohol in a number of biochemical reactions has been studied principally in rats by the use of isotopically tagged compounds as indicated in the preceding paragraph These studies have implicated both vitamin B_{12} and folic acid (in its 'biologically active form folinic acid) as coenzymelike factors in many of these chemical reactions The exact mode of action of the two vitamins in these processes and the significance of the processes to the normal animal are not yet clear but it is apparent that the close association of vitamin B_{12} and pteroylglutamic acid in hematopoiesis is paralleled by a similar close relationship in the intermediary metabolism of one carbon fragments So close is this relationship that it has been suggested that one of the functions of vitamin B_{12} is the conversion of folic acid into its metabolically functional form folinic acid (80) Research is proceeding so rapidly in this and related fields that there is little doubt that within a few years a much clearer picture of the precise metabolic roles of vitamin B_{12} will have emerged For a detailed presentation of the position at the time of writing the reader is referred to the text (134) mentioned earlier

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CHAPTER 6

NICKEL

The first recognition of nickel as a constituent of the ash of plants occurred a century ago (8) but almost seventy years passed before its universal distribution in soils plants and animals began to be recognized Bertrand and co workers (4) in France McHargue (15) in the United States and Berg (3) in Germany were the earliest workers to examine a wide range of soils plants and human and animal tissues for nickel They showed that this element normally occurs in much higher concentrations than cobalt in soils and plant tissues and in much lower concentrations than cobalt in animal tissues This finding has been confirmed by subsequent investigations employing more delicate methods of analysis but the concentrations of nickel in most animal tissues and fluids are so low that accurate estimation with the available analytical techniques is extremely difficult.

The demonstration of the presence of nickel in plant and animal tissues has not been accompanied by any conclusive demonstration of a physiological role for this element So far nickel has not been shown to play any part in the nutrition of plants or animals nor have any diseases in plants or animals been found to be due to nickel deficiency or to respond to nickel supplements It must be pointed out however that no serious attempts to demonstrate an essential function for nickel with the aid of modern experimental techniques have yet been undertaken This is surprising in view of the closely similar physical and chemical properties of nickel and cobalt and the reasonable expectation that they might have some physiological properties in common Some results were obtained which indicated that nickel might partially but only to a limited extent replace cobalt in the treatment of cobalt deficiency of sheep when suboptimal levels of cobalt were fed (5 7) but this has apparently not been confirmed by later work (1) and nickel supplements without cobalt or when adequate cobalt is administered have been found of no benefit in the treatment of any of the wasting diseases of sheep and cattle

Studies of the effect of nickel on various biological systems have been made which indicate that Ni^{++} activates arginase (10) carboxylase (19) and trypsin (22) *in vitro* and inhibits acid phosphatase under certain conditions (17) Whether these effects are of any significance in the living organism is unknown but it is probable that the toxicity of

nickel to animals discussed below is due to inhibition of enzyme systems by this element

I Nickel in the Body

Acceptable data on the nickel content of the tissues and fluids of man and the higher animals are so few that the normal distribution of this element in the animal body cannot be given In 1929 nickel was reported to be a normal constituent of bones (14) and later evidence has revealed that this element can accumulate in considerable quantities in the skeleton Phatak and Patwardhan (18) fed rats for 8 weeks on normal diets supplemented with 3 different forms of nickel, each at levels of 25 50, and 100 mg nickel per 100 g of diet, and determined the distribution of the retained nickel in the tissues No nickel could be measured with the particular method used in the tissues of the rats receiving only the basal diet but in all the tissues examined of those animals which received the nickel supplements appreciable quantities were found At each level of nickel intake the bones (femora) contained far higher concentrations of nickel than any other tissues, indicating that the skeleton is the main storage organ of the body for this element (Table 22) The level of nickel in the rest of the tissues as well as in the bones, varied in general with the level of intake but it is of interest to note that in contrast to the position with most of the trace elements the liver has apparently little capacity to store nickel

A further unusual feature revealed by the data of Table 22 is the very high nickel content of heart muscle at the higher nickel intakes High intakes of other trace elements are not accompanied normally by greatly increased concentrations of these elements in either heart or skeletal muscle

The distribution of nickel in the tissues of the mouse, following intraperitoneal injection of a small nontoxic dose of radioactive Ni^{63} as nickel chloride has recently been studied (23) Wide distribution of the administered nickel throughout the tissues was found with the kidneys, lungs and plasma containing the highest concentrations and the brain and muscles the lowest The retained nickel disappeared rapidly from all tissues with the exception of the lungs and the brain

The values for nickel appearing in Table 22 are all many times higher than the few recorded for the tissues of other species consuming normal diets Thus Bertrand and Macheboeuf (4) found 0 09 and 0 12 p p m nickel for fresh human and ox liver respectively, and 0 04 and 0 13 p p m for fresh human and ox pancreas They also found 0 004 p p m in cow s milk and 0 02 p p m in egg yolk These very low levels of nickel in

TABLE 22

DISTRIBUTION OF NICKEL IN THE TISSUES OF RATS AT HIGH NICKEL INTAKES[a]

Basal diet plus nickel carbonate	Bones	Liver	Kidney	Spleen	Heart	Intestine	Testes	Blood	Skin
25 mg Ni per 100 g diet	187.6	1.0	11.9	5.5	7.9	18.4	13.2	4.8	2.4
	135.2	1.5	10.0	2.6	7.6	6.9	23.3	3.4	1.0
50 mg Ni per 100 g diet	288.9	13.2	11.5	28.2	20.3	21.8	58.9	10.1	8.4
	228.8	4.3	30.6	33.6	12.4	15.0	5.9	13.0	3.6
100 mg Ni per 100 g diet	358.5	11.1	43.1	28.6	50.1	25.4	18.6	31.0	12.5
	346.9	13.0	33.7	33.1	40.0	25.6	26.8	21.1	7.1

[a] Measured in p.p.m. Ni of *fresh* tissue (18).

metal) are now much more widely used in modern food and beverage industries than pure nickel and are much more resistant to corrosion The use of nickel catalyst in the hydrogenation of oils also results in some contamination of the finished product with this element but the amount is normally very small Over 100 samples of refined hydrogenated edible fats were recently analyzed for nickel in India and the highest value found in any sample was only 0 4 p p m (18)

IV Nickel Toxicity

Nickel is a relatively nontoxic element when ingested Its toxicity is less than that of cobalt and of the same order of that of zinc A very large amount of investigative work has been carried out with nickel stimulated principally by the practical question as to whether or not a toxic amount of nickel is taken up by foods cooked in nickel utensils Much of this work was done towards the end of the last century prior to the development of a satisfactory method for the estimation of small amounts of nickel As a result the data are confused, and often discordant but they leave little doubt that the amounts so ingested are certainly not harmful to man in spite of the fact that foods especially acid foods take up nickel during cooking in nickel utensils (6) There are reports of whole families using only nickel utensils for the cooking and handling of their food for periods of months and even years without any apparent damage to health. Some of the corroded nickel, which incidentally is much less than the amounts of tin or aluminum corroded from utensils made of these metals, is undoubtedly absorbed but the amount is exceedingly small and causes no observable damage (6 18) It appears therefore that nickel contamination of foods does not present an appreciable health hazard

Mention should be made however of the high incidence of respiratory tract neoplasia and skin ailments among workers in nickel refineries (20) and of the fairly recently reported carcinogenetic property of nickel (11)

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metal) are now much more widely used in modern food and beverage industries than pure nickel and are much more resistant to corrosion The use of nickel catalyst in the hydrogenation of oils also results in some contamination of the finished product with this element but the amount is normally very small Over 100 samples of refined hydrogenated edible fats were recently analyzed for nickel in India and the highest value found in any sample was only 0 4 p p m (18)

IV Nickel Toxicity

Nickel is a relatively nontoxic element when ingested Its toxicity is less than that of cobalt and of the same order of that of zinc A very large amount of investigative work has been carried out with nickel stimulated principally by the practical question as to whether or not a toxic amount of nickel is taken up by foods cooked in nickel utensils Much of this work was done towards the end of the last century prior to the development of a satisfactory method for the estimation of small amounts of nickel As a result the data are confused, and often discordant, but they leave little doubt that the amounts so ingested are certainly not harmful to man in spite of the fact that foods especially acid foods, take up nickel during cooking in nickel utensils (6) There are reports of whole families using only nickel utensils for the cooking and handling of their food for periods of months and even years without any apparent damage to health Some of the corroded nickel which incidentally is much less than the amounts of tin or aluminum corroded from utensils made of these metals, is undoubtedly absorbed but the amount is exceedingly small and causes no observable damage (6 18) It appears therefore that nickel contamination of foods does not present an appreciable health hazard

Mention should be made however of the high incidence of respiratory tract neoplasia and skin ailments among workers in nickel refineries (20) and of the fairly recently reported carcinogenetic property of nickel (11)

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tacular improvement and productivity In addition, studies of the metabolism of zinc deficient plants disclosed a number of biochemical lesions which helped to establish the fundamental importance of zinc in all living cells and stimulated a search for similar biochemical defects in the zinc deficient animal

II Zinc in Animal Tissues and Organs

1 *The Zinc Content of the Whole Body*

The body of a normal 70 kg man has been estimated to contain a total of some 2 2 g zinc, which gives an over all concentration of about 30 p p m (56) This is very close to one half the amount of total body iron but is 5 times that of nonhemoglobin iron and 10 to 15 times that of copper Similar high concentrations of zinc occur in the whole bodies of various other mammals (56 82) (Table 23) Newborn and very young mammals are apparently more variable than adults in zinc content and in contrast to the position with copper they tend to contain somewhat lower or similar, concentrations of this element at birth and at maturity (Table 24) The high concentration of zinc in the newborn mouse compared with those of the other newborn mammals included in Table 24 is difficult to understand and is not entirely supported by the analyses of Nishimura (62) of 9–13 days old mice The comparatively high values for the newborn cat and guinea pig can probably be accounted for by the fact that they are born covered with hair whereas the other species are born naked Similarly the relatively high value for the adult rabbit can no doubt be explained by the large amount of fur carried by this species Hair and fur as will be shown contain much higher concentrations of zinc than almost all other body tissues

There is no evidence of any appreciable fetal storage of zinc as occurs with iron and copper The concentration of zinc in the human fetus changes little throughout fetal life and the liver and spleen together always contain approximately the same proportion (roughly one quarter) of the total zinc (94) No such data are available for other species but in the cat and pig there is no fall in the whole body concentration of zinc during suckling and in the rat and the pig there is a substantial *rise* from the newborn levels during suckling and the early stages of growth followed by a later fall to adult values (82) Of greater significance is the fact that the liver and spleen of the rat, rabbit, and pig tend to contain higher concentrations of zinc at the end of suckling than at birth Only the kitten which has comparatively high levels of zinc in these organs at birth reveals a fall during suckling In all these species however the levels of zinc in these organs fall when mixed feeding begins

TABLE 23
THE ZINC CONTENT[a] OF TISSUES

	Rat			Cat			Man
	Normal		Added zinc	Normal		Added zinc	Normal
	(Lutz)	(Drinker)	(Drinker)	(Lutz)	(Drinker)	(Drinker)	(Lutz)
Blood	6.7	7.0	15.0	4.6	2.0	10.0	5.2
Gastrointestinal tract	15.1	45.0	60.0	19.1	25.0	50.0	11.3
Pancreas	—	17.3	19.0	24.8	27.0	330.0	12.4
Liver	20.7	47.0	50.0	41.1	55.0	340.0	54.9
Kidney	14.4	50.0	110.0	14.1	14.0	220.0	35.0
Spleen	36.3	228.0	250.0	12.6	33.0	35.0	11.3
Lung	23.6	48.0	60.0	14.5	28.0	30.0	6.8
Testes	11.9	82.0	—	8.5	—	—	308.9
Brain and spinal cord	13.4	53.0	40.0	9.4	20.0	20.0	8.3
Muscle	13.6	38.0	30.0	21.1	22.0	25.0	30.2
Hide	33.8	36.0	50.0	39.1	30.0	60.0	—
Hair	—	—	—	224.1	—	—	163.0
Bone	178.3	—	—	125.9	227.0	260.0	100.8

[a] Measured in p.p.m. of fresh tissue.

(82) It appears, therefore, that the young mammal can readily obtain its zinc requirements from the maternal milk, provided that the colostrum which is 4 to 5 times richer in zinc than later milk is available to it It has been demonstrated that when the newborn mouse is denied the colostrum by being fostered onto mothers at a later stage of lactation a pronounced reduction in the concentration of zinc occurs in the whole body (62)

TABLE 24

CONCENTRATION OF ZINC[a] IN THE WHOLE BODIES OF MAMMALS

	Human	Pig	Cat	Rabbit	Guinea pig	Rat	Mouse
Newborn[b]	19	10	29	23	35	24	46
Adult[c]	28	25	23	*50*	–	*30*	–

[a] Measured in p p m Zn of fat free body tissue
[b] Widdowson (93)
[c] Spray and Widdowson (82)

2 *The Zinc Content of Various Tissues*

Zinc has been found in all the tissues and organs of the body that have been examined and there is every reason to believe that it occurs in all living cells The zinc content is invariably substantially higher than the copper content although the ratio of zinc to copper is lower in the brain and higher in the muscles than in other organs From the rather limited range of data available it appears that species differences among mammals are small In most cases the hair the pigmented tissues of the eyes, and the bones carry far higher concentrations of zinc than the other body tissues and organs The values for the rat cat and man obtained by Lutz (56) and by Drinker and Collier (27) in their extensive pioneer investigations of zinc as an industrial health hazard given in Table 23 and taken from the review by Hegsted *et al* (43) illustrate these points

These findings have been reasonably well substantiated by more recent studies Thus Eggleton (30) obtained the following mean values for the organs of 26 Chinese subjects liver 245 muscles 226 kidneys 186 pancreas 135 heart 100 adrenals, 82 spleen 72 lungs 67 cerebellum 55, and cerebrum 43 p p m zinc on the *dry* basis Individual variability was considerable in the liver kidneys and muscles but small in the other organs Data for the concentration of zinc in the tissues of the ruminant are exceedingly scanty A mean of 125 p p m zinc on the dry basis (range 106–170) was obtained for the livers of 30 milking cows (37) and the following values for the organs of a single sheep kidney 90 intestine 81 spleen 76 adrenals 74 pancreas 72 brain 68 lung 64

heart 62 liver 54 and sternum 51 p p m zinc on the dry *fat free* basis (51)

Zinc appears to resemble lead and nickel in its tendency to accumulate in the bones as a number of workers have reported very high values (150–250 p p m) for bones and teeth (20, 27 29 56) In view of the large mass of bone an appreciable proportion of the total body zinc must be present in this site In haired furred or wooled animals a large proportion of the total zinc must reside in the hair fur or wool In the rat and the hedgehog no less than 38% of the whole body zinc has been found to be situated in the skin and hair or the skin and bristles (82) This can hardly be regarded as a store" of zinc however as it is difficult to imagine much of it finding its way back into the body for use in the various enzyme systems in which this element is involved The zinc content of epidermal structures is discussed later

Unlike iron and copper zinc is not normally stored in large amounts in the liver Indeed it seems that the rat has a very limited capacity to store zinc in any of its organs except perhaps the bones In the cat on the other hand the feeding of large amounts of zinc results in markedly increased concentrations of this metal in the liver spleen pancreas and bones (Table 23) Rats fed zinc deficient diets contain much less total zinc in their bodies than normal animals (84) but this appears to be due to their reduced size as a result of the zinc deficiency According to a recent report the tissues of severely zinc deficient rats greatly dwarfed in size contain closely similar *concentrations* of zinc to those of control rats receiving adequate zinc and being normal in size (19)

The zinc content of the pancreas has excited some interest since the claim that the pancreas of diabetics is significantly lower in zinc than that of nondiabetics (75) This has not been confirmed by later work in which little difference was found when the results were expressed on the basis of fat free pancreas tissue (33) The actual mean figures were 30 6 μg Zn per g for 27 nondiabetics and 25 3 $\mu g/g$ for 21 diabetics The relation of zinc to insulin action is considered more fully later

The significance of the very high concentration of zinc in the testes of man given in Table 23 is unknown but appears worthy of further study Some high values for the zinc content of the testes of rams have also been reported and it was shown earlier that during the period of sexual activity of the herring the testes of the male contained twice as much zinc as the rest of the body (8) During the same period practically no difference was found in the zinc content of the ovaries and of the remainder of the body These facts may be related to the male

infertility, and to the reduction in size of the testes, but not of the ovaries which was demonstrated in rats fed zinc deficient diets (18) It is tempting to suggest that zinc is concerned in some way in testicular development and function, but much more evidence must be obtained before such a suggestion can be either substantiated or disproved

3 *Zinc in Epidermal Structures*

Epidermal structures have been known to contain particularly high concentrations of zinc since the work of Lutz (56) Whether it performs any useful function in these tissues or how it is combined with the proteins such as keratin, or with other compounds present is not known, but it is probably pertinent that alopecia and severe skin lesions are specific characteristics of zinc deficiency in rats and mice Eggleton (31) has produced evidence pointing to an interesting relationship between beri beri and the zinc content of human epidermal structures The subnormal levels of zinc found in these structures (hair, nails and skin) in beri beri sufferers are regarded as a reflection of low intakes of zinc in beri beri producing diets because of a reputed high correlation between the zinc and thiamin content of foods (31) Conclusive evidence from elsewhere of a significant reduction in the concentration of zinc in epidermal tissues or in other tissues of the body as a result of zinc deficiency is lacking

Representative values for the zinc content of skin hair nails bristles and wool are presented in Table 25 The figures for wool are of some interest as the sheep is normally shorn annually Assuming the removal of 8–10 lb wool at each shearing the sheep would lose as much as 400–600 mg zinc each year This amount of zinc as is pointed out later would be ingested from normal grazings within a few days or at most 2–3 weeks

4 *Zinc in Eye Tissues*

Extremely high concentrations of zinc as well as of copper occur in the eye tissues especially the pigmented portions of a wide range of animal species In fact the highest known concentrations of zinc in living matter occur in the melanin pigmented tissues of the eyes of some fresh water fishes (11 54) In mammals amphibians and salt water fishes the concentrations are a good deal lower but they are always higher than those in the unpigmented tissues of the same animals (13) Considerable species and individual differences exist but the various eye tissues can be placed in the same order in respect to zinc concentration in each of the species examined This order in descending con

centrations of zinc which is similar to but not identical with that given earlier for copper, is as follows iris choroid (plus pigment epithelium), retina (minus pigment epithelium) lens, aqueous and vitreous humor, sclera cornea and optic nerve Values as high as 436 and 277 p p m zinc on the dry basis have been reported for the iris and choroid respec

TABLE 25
ZINC CONCENTRATIONS[a] IN EPIDERMAL STRUCTURES

	Healthy adults			Beri beri sufferers		
	Number of samples	Mean	Range	Number of samples	Mean	Range
Head hair[b]	13	255	84–444	7	173	110–229
Pubic hair[b]	8	197	71–342	4	83	45–115
Fingernails[b]	13	195	121–260	13	88	22–184
Toenails[b]	11	198	96–340	6	90	31–153
Skin[b]	8	26	12–55	7	13	6–21
Human hair[c]	—	215	—	—	—	—
Rat skin and hair[c]	—	47	—	—	—	—
Hedgehog skin and bristles	—	49	—	—	—	—
Sheep s wool[c]	—	164	—	—	—	—
Rat hair[d]	—	140	—	—	—	—
Sheep s wool[d]	—	—	70–120	—	—	—

[a] Measured in p p m of fat free dry tissue
[b] Eggleton (31)
[c] Spray and Widdowson (82)
[d] Jones (51)

tively in sheep s eyes and of 246 and 139 p p m for these tissues in the eyes of cattle Levels nearer those of normal unpigmented tissues of the body were obtained for such eye tissues as the cornea and optic nerve The eyes of colored and albino rabbits were also examined Zinc concentrations of 127 and 466 p p m were found in the iris and choroid respectively of the former animals compared with 54 and 86 p p m for these tissues in the albinos (13) It was shown further that the zinc (and other metals present) occurred in nondialyzable form in melanin protein fractions of the eyes and that these fractions contained most of the zinc in the pigmented tissues

These highly interesting findings invite speculation as to the physio logical function of the zinc copper and other metals There is much evidence as Bowness and Morton (12) have pointed out that a number of metals combine with and perhaps influence the color of natural melanin complexes although melanins can be formed without any

In the gastric mucosa, carbonic anhydrase probably serves an indispensable function in catalyzing the rate of hydration of carbon dioxide for the neutralization of the residual excess alkalinity from the secretion of hydrogen ions in the production of hydrochloric acid The fundamental reaction in the production of gastric hydrochloric acid is believed to be the ionization of water The secretion of hydrogen ions leaves a residual excess alkalinity which is neutralized by carbon dioxide and it has been suggested that the uncatalyzed rate of hydration of carbon dioxide (i e in the absence of carbonic anhydrase) would not be fast enough to neutralize this residual alkalinity (22) It is possible that carbonic anhydrase has a similar indispensable function in the secretion of other acid and alkaline juices in the body such as the pancreatic juice, but there is as yet no proof of this

Similarly, it seems likely that carbonic anhydrase plays a role in the calcification of bone and in the formation of egg shells High concentrations of this enzyme have been recorded in that portion of the oviduct of hens in which shell formation takes place

III Zinc in Blood

The zinc content of the whole blood of mature cattle and sheep generally lies between 2 5 and 3 5 μg /ml (37, 51) Adult rabbits have been reported to contain 2 5 μg /ml of plasma and 9 μg /ml of corpuscles (4) Values reported for whole human blood range from 3 to 9 μg Zn per ml, with a high proportion lying between 5 and 7 μg A higher mean value for normal human blood is given by Vallee and Gibson (88) who used an accurate improved dithizone method They obtained the following mean concentrations for whole blood and its component parts whole blood 8 8 ± 2 0 μg Zn per ml, plasma, 3 0 ± 1 6 μg /ml, packed erythrocytes 14 4 ± 2 7 μg /ml erythrocytes $1\ 34 \pm 0\ 2 \times 10^{3}$ μg per million cells leucocytes $3\ 2 \pm 1\ 3 \times 10^{2}$ μg per million cells Calculations showed that 75% of the total zinc in the blood was situated in the erythrocytes 22% in the plasma and 3% in the leucocytes The individual leucocyte however contained 25 times as much zinc as the individual erythrocyte

The concentration of zinc in the peripheral leucocytes of patients with chronic myelocytic lymphocytic, and monocytic leukemia has been shown to be greatly reduced below normal and not to be raised by injections of stable zinc gluconate (38) A rise to normal levels occurs in clinical remission and under therapy with X rays or urethane accompanying the falling leucocyte count (38) A three fold to ten fold elevation of the zinc content of the leucocytes has been noted in patients

with "refractory anemia" accompanied by leukopenia with concentrations of white cells below 2000 per mm³ of blood (39) The physiological significance of the zinc in leucocytes and of the profound changes in the concentrations which occur is unknown but a great deal has been learned of its chemical associations in the cell It does not occur in leucocytes as carbonic anhydrase since carbonic anhydrase activity has not been found in these cells (55) In human leucocytes zinc occurs in constant proportion to total content of protein and is present in firm combination with protein as two types of zinc protein complexes—one type soluble in a phosphate buffer solution of pH 7 2 and the other insoluble in this fluid (45) Further purification has resulted in the preparation of leucocyte proteins having a maximum zinc to protein ratio of *3 mg Zn per g protein which is comparable in magnitude to the metal* contents of other metalloproteins (90)

Under normal conditions the whole of the zinc in erythrocytes can be accounted for as carbonic anhydrase (48) Long continued administration of large (250 mg daily) oral doses of zinc to rabbits has been reported to induce increased concentrations of zinc in the red corpuscles which were not accompanied by increases in the carbonic anhydrase of these cells (4) but this finding needs confirmation In almost all patients with anemia other than pernicious anemia both the zinc and carbonic anhydrase activity of blood are lowered in parallel fashion so that the decreases are proportional to the decreases in hematocrit hemoglobin levels and erythrocyte counts (89) The zinc and enzyme values *per unit of R B C* remain in the normal range (89) Pernicious anemia patients on the other hand show no such decrease in absolute values for zinc and carbonic anhydrase in spite of marked lowering of hematocrit hemoglobin levels and erythrocyte counts (89) The zinc and enzyme values *per unit of R B C* in this disease are therefore significantly elevated above normal This occurs even when the zinc concentration is calculated per million cells so that the increased red cell size in pernicious anemia is eliminated as a contributing factor

It is impossible to evaluate the physiological significance of these findings at present or to know how far they are applicable to other species It would be of great interest for instance to compare the zinc and carbonic anhydrase activity of the red cells of the blood of normal sheep and of sheep suffering from the anemia of cobalt deficiency Vallee and Gibson (89) have suggested as a result of these findings that "the hemoglobin and carbonic anhydrase systems are structurally discrete though functionally related" and they indicate that erythrocyte zinc concentration might be used as a relatively simple index of the carbonic

anhydrase content in studying various phases of respiratory physiolog
In this connection it should be mentioned that newborn babies hav
been observed to contain much lower concentrations of corpuscular zin
than their mothers Berfenstam (4) reported levels of 347 μg % in infant
at birth which rose steadily during the first few months of life to reac
very close to 1000 μg % at about one year of age and adult values c
1244 μg % at puberty The same worker reported a somewhat simila
position in rabbits, in which values of 528 μg % of zinc were observed i
the erythrocytes of newborn rabbits and 900 μg % in adults of this specie
During this period of growth the plasma zinc concentrations were foun
to *fall* very slightly in the human species and markedly in the rabbit (4)

In contrast to the position with iron and copper little is yet know
of the form in which zinc exists in plasma It seems very probable tha
it occurs in combination with one or more of the plasma proteins, es
pecially as the plasma proteins are readily precipitated by zinc and th
urine contains so little zinc except in patients with albuminuria Th
zinc in plasma does not exist as carbonic anhydrase, since no carboni
anhydrase activity can be demonstrated in this fraction of the blood (55)
The level of zinc in plasma appears however, to vary with age and t
be fairly readily increased by oral or parenteral administration of larg
doses of zinc The fall in plasma zinc from the newborn to the adul
level in humans and rabbits has already been mentioned The differenc
between fetal and maternal human blood plasma is even greater Dat
from 10 fetuses at 100–150 days revealed a mean plasma zinc concentra
tion 3 times that of adult values (4) Large oral doses of zinc signifi
cantly increase whole blood zinc in rats and cats (Table 23), presumabl
largely as a result of a rise in plasma zinc and they induce large increase
in plasma zinc in rabbits to levels as high as 20–30 μg /ml (4) These
increases in plasma zinc as mentioned previously, are accompanied by
increased corpuscular levels of this metal A single intravenous injection
of 30 mg of zinc into rabbits was followed by an immediate rise in
plasma zinc concentration to a level of 150 μg /ml (4) The effect of
zinc deficient diets on plasma zinc levels has not, so far as is known
been determined but it is significant that the feeding of such diets does
not result in an appreciable lowering of the carbonic anhydrase activity
of the blood of rats even when these animals are *in extremis* from zinc
deficiency (19 48)

IV The Zinc Content of Milk

The normal range of concentration of zinc in the milk and colostrum of the cow ewe and woman has been fairly extensively studied Individual variability from animal to animal appears to be somewhat smaller than with other trace metals and it is clear that milk is unusually rich in this element It was in fact the observation that cows milk is considerably richer in zinc than in iron or copper and very many times richer than in manganese which was largely responsible for stimulating the original Wisconsin attempts to demonstrate an essential role for zinc in mammalian nutrition The very high concentration of zinc in cows milk compared with that of other trace elements that have been shown to be essential in nutrition is illustrated in Table 26 which gives what appears to be the most acceptable range of values reported for these metals

TABLE 26

NORMAL RANGE OF CONCENTRATION OF VARIOUS TRACE ELEMENTS IN COWS MILK[a]

Zinc	Iron	Copper	Iodine	Manganese	Cobalt
3000–5000	300–600	50–150	30–70	20–30	0.4–0.7

[a] Measured in μg/liter

Species differences in the zinc content of cows ewes and human milk either do not exist or are very small Sato and Murata (72) were unable to detect significant differences between these species and found a high proportion of the samples of each to lie between 3 and 4 mg Zn per liter Berfenstam (4) obtained values mostly lying between 3 and 5 mg/l for both human and cows milk and Archibald (2) reported a mean of 3.9 mg/l for normal cows milk. Supplementing the normal rations of these cows with a zinc salt consistently raised the zinc content of the milk to a mean of 5.1 mg/l A similar increase in the zinc content of the milk of rabbits to levels above normal by relatively massive oral dosage with zinc salts has also been reported (4) Whether subnormal intakes of zinc by lactating animals result in subnormal concentrations of zinc in the milk as occurs with copper under conditions of copper deficiency has not been determined

Similarly there appears to be no conclusive evidence of a fall in the zinc content of true milk throughout lactation as has been shown to take place with copper There is no doubt however that the zinc content of colostrum in all species studied is some 3 to 5 times that of later milk. Values for both human and cows colostrum as high as 20 mg Zn per liter have been reported (4) Evidence of the importance of

the colostrum as a source of zinc to the newborn mouse is discussed in the following section

V Zinc Deficiency in Animals

1 *Sheep and Cattle*

Zinc deficiency has never been observed in man or in farm stock under either naturally occurring or experimental conditions It seems very unlikely that such a deficiency could ever arise in grazing or stall fed sheep or cattle because of the normal high content of zinc in plant materials Pasture plants and forages usually contain 30–100 p p m zinc on the dry basis (3) when grown on ordinary soils In the con siderable areas of zinc deficient soils that exist in different parts of the world the growth of crops and pastures may be greatly reduced various specific zinc deficiency symptoms may develop in the plants and the plants also usually carry subnormal concentrations of this element All of these difficulties can readily be prevented or overcome by treatment of the soils or plants with zinc containing fertilizers or sprays The effect of the zinc deficient soils is much more pronounced on the growth of the plants, i e on the bulk of herbage produced than on its zinc content and even under the most acutely deficient conditions the concentration of zinc in pasture plants rarely, if ever falls below about 10 p p m and in cereal grains below about 5 p p m In these circumstances it is not surprising that zinc deficiency in grazing stock has never been observed The amounts of zinc ingested by grazing sheep and cattle under any conceivable conditions would therefore be very much greater per unit of body weight, than those shown to be near the minimum for small laboratory animals

2 *Pigs and Poultry*

There do not appear to have been any attempts to induce zinc defi ciency in pigs or poultry by the use of specially purified diets A defi ciency of this element under any reasonable practical conditions seems extremely unlikely because the cereal grains and their by products which form the basis of most rations for these species contain liberal amounts of zinc Whole cereal grains normally contain 20–80 p p m and the bran and germ as much as 150 p p m (3)

The question of supplies of zinc for laying hens is of some interest because egg yolk contains appreciable but very variable amounts and heavy egg production involves a considerable loss of this element in laying birds Romanoff and Romanoff (67) give 700–1000 μg and 7 μg as the average zinc contents of egg yolk and egg white respectively

and state that this metal is only occasionally present in the shell Later work with a highly accurate method (87) similarly demonstrated little or no zinc in egg whites and shells In the yolks of 7 eggs from normal hens 198–978 (mean 555) μg Zn was found and 277–1040 (mean 581) μg Zn in the yolks of 7 eggs laid by a hen injected with zinc glycine (20 mg Zn) during the period 1–10 days following injection The laying pellets consumed by these birds contained 32 p p m Zn and the average total zinc intake per bird almost wholly from the pellets was 5 7 mg per day The authors raise the question as to whether these amounts of zinc in the yolk are merely to provide a store for the needs of the developing chick or whether the egg serves as a means of excreting excess zinc No information on the influence of varying intakes of dietary zinc on the zinc content of the egg or on absorption and excretion of zinc in birds have so far as is known been carried out

3 *Rats and Mice*

Zinc deficiency has been induced in rats and mice by the feeding of specially purified diets supplemented with vitamins and other minerals (18 19 25 35 47 48 62 84 86) There has been a steady improvement in these diets in terms of degree of zinc deficiency combined with a capacity to promote growth when supplemented with zinc since the original work establishing zinc as an essential element for growth in the rat (86) Zinc deficiency symptoms have been demonstrated in rats on diets estimated to contain not more than 1 6 p p m (47 86) and in mice to contain as little as 0 3 p p m zinc (25) In this latter investigation 18% of the zinc deficient mice died within 8 weeks although 100% of the controls on the same diet plus zinc survived More recently diets have been compounded supplying less than 1 μg Zn per day to rats or below 0 2 p p m on ordinary intakes (19) The achievement of such extremely low concentrations of zinc in diets otherwise satisfactory for growth represents an important step forward in the study of zinc deficiency and it is unfortunate that the details have not yet been reported Special treatment (hydrolysis followed by dithizone extraction) of the protein components of the diets appears to have been the most significant of the devices used to eliminate zinc Eggleton (31) showed many years earlier that most of the zinc occurring naturally in foodstuffs is associated with the protein containing tissues

In addition to the use of such diets zinc deficiency has apparently been demonstrated relatively simply in baby mice merely by exclusion of zinc rich colostrum from their diets by fostering them at birth with mothers at later stages of lactation (62) Newborn mice nursed by their

own mothers for 2½–4 days before transfer to foster mothers grew normally whereas newborn mice that were fed for periods of 3–5 days by foster mothers that had been lactating for 13–18 days and were then returned to their own mothers usually developed disorders somewhat typical of zinc deficiency which could be prevented or which responded to oral administration of zinc

The zinc deficiency syndrome in both rats and mice is characterized clinically by retardation or failure of growth moderate inappetence and alopecia and pathologically by gross epithelial especially cutaneous lesions The effect on growth is dependent upon the degree of deficiency of zinc in relation to the growth promoting capacity of the diet in other respects The reduction in food consumption is of itself, insufficient to account for the impaired growth (84) indicating that inappetence is not a dominant feature of zinc deficiency in these species and that there is an inefficient utilization of the food consumed The alopecia and cutaneous lesions are not immediately revealed because the zinc deficient animal makes over all growth only to the limit permitted by its zinc intake Thus on the acutely zinc deficient diets mentioned previously, which supplied less than 1 μg Zn per rat per day, growth ceased abruptly and the animals remained dwarfed until their integument broke down (19) Marked retardation or complete failure of growth occurred also in earlier experiments with rats and mice on less severely zinc deficient diets accompanied in most, but not all cases by alopecia and pathological changes in the integument after several weeks on the deficient diet

The histopathology of zinc deficiency in rats has been studied by Follis and co workers (35) and summarized in the following terms extreme parakeratosis of the esophagus with a thick layer of keratinised cells hyperkeratinisation of the skin with thickening of the epidermis and loss of hair follicles but not of sebaceous glands Vascularization of the cornea and leucocyte infiltration reminiscent of riboflavin deficiency occur in some animals Similar changes were observed in zinc deficient baby mice but, in addition there was a retardation of ossification, an accelerated eruption of the incisors and separation of the eyelids a rosary like tail, clubbed digits and deformed nails (62)

There is some disagreement on the influence of zinc deficiency on the levels of zinc in the whole body and its tissues Several groups of workers have reported subnormal concentrations of zinc in the whole bodies of deficient rats (84 86) and mice (35 62) and in the bones of rats (35), but in one brief report of severe zinc deficiency in rats (19) it was claimed that all tissues when compared on a weight for weight

basis with those from rats receiving adequate zinc and growing normally contained closely similar concentrations of zinc

The question as to whether lack of zinc specifically affects reproductive function is not yet fully resolved Follis and co workers (35) observed in male rats autopsied after 61 and 74 days on a zinc deficient diet numerous atrophic semeniferous tubules lined only by spermatogonia and in zinc deficient females no corpora lutea in the ovaries with partial follicular development and a keratinized vaginal epithelium These workers attributed these changes to inanition, rather than directly to zinc deficiency Colmano and Fiori (18) on the other hand found the female reproductive organs to be normal in their zinc deficient rats whereas the male gonads were underdeveloped and matings were infertile The relation of zinc deficiency to sex function merits further study especially in view of the occasional very high values obtained for the zinc content of the testes to which earlier reference was made

4 *Mode of Action of Zinc*

Various attempts have been made to determine the primary metabolic defects responsible for the seriously impaired growth and pathological effects of zinc deficiency The nature of the corneal and cutaneous lesions of zinc deficient animals can be compared in certain respects with those occurring in rats and mice following deprivation of a number of nutrients including vitamin A riboflavin biotin pantothenic acid pyridoxin and essential fatty acids and it can be postulated that zinc is concerned in some way with the utilization of these nutrients Such a relationship has already been hypothecated for zinc and riboflavin (35) but no supporting experimental evidence has yet been adduced Particular attention has been devoted to the status of various enzyme systems in the blood and tissues of zinc deficient animals especially to those enzymes of which zinc is known to be a component such as carbonic anhydrase or which it has been shown to activate such as aldolase uricase and kidney phosphatase The role played by carbonic anhydrase in the body has already been discussed

The carbonic anhydrase activity of the blood and tissues of zinc deficient rats is not appreciably reduced below normal even when the rats are *in extremis* during the terminal stages of the deficiency (19 18) The liver uricase activity of zinc deficient rats is also not reduced under such conditions although there is a rise in plasma uric acid (92) There has been demonstrated however a lowering of kidney phosphatase activity (47) and of brain aldolase activity (19) in rats and a marked reduction in liver and kidney catalase activity in mice (25) Thus reduc

tion in catalase activity must be an indirect action of zinc because the addition of zinc salts to tissue preparations from zinc deficient mice does not increase the catalase activity Nor is there any evidence that this enzyme contains zinc

These findings reveal clearly that zinc is essential for the proper functioning of several enzyme systems in the body but the biochemical lesions so far disclosed can hardly be sufficient, in magnitude or scope, to account for the profound effects of zinc deficiency in the animal Serious impairment of growth occurs before any reduction in the activity of any enzymes so far studied can be detected and greater than could be accounted for by the reduction in food consumption It has recently been claimed that this is associated with a rise in metabolic rate and consequent decrease in efficiency of food utilization (19a), but the exact means by which this is brought about is unknown

In green plants and fungi a shortage of zinc is also associated with retarded growth and morphological changes in the organized structures which suggest serious metabolic disturbances The retardation in growth which occurs in fungi on zinc deficient media has been claimed to be due to a failure of carbohydrate metabolism consequent upon a break of one or more links in the chain of anaerobic glycolysis (36) An impairment of carbohydrate metabolism has also been reported in zinc deficient higher plants (66) and a material decrease in the aldolase activity of tissue suspensions of such plants has recently been demonstrated (63) Convincing evidence of a disturbance of carbohydrate metabolism in zinc deficient animals has yet to be obtained but suggestive evidence of some relationship between zinc and vitamin B_1 has been brought forward by several workers Bertrand and Bhattacherjee (6) claim that in zinc depleted rats the metabolic functions of this vitamin are suppressed Imada (50) reports a synergistic action between zinc and vitamin B_1 in rabbits and Nishimura (62) found that administration of this vitamin had a mitigating influence on zinc deficiency in baby mice It would seem that the whole question of carbohydrate metabolism in zinc deficient animals merits further critical study

Numerous claims have been made that zinc influences carbohydrate metabolism through its relation to insulin There is no doubt that the addition of zinc in insulin solution causes a delay in its physiological action and prolongs the hypoglycemia but there is still no conclusive evidence that this element plays any part in the normal production or action of insulin *in vivo* Pure amorphous preparations of insulin have been crystallized with salts of radioactive zinc and 0 31% and 0 36% of zinc found in the crystalline products formed (17) Other studies with

radioactive zinc have shown that the pancreas at least in dogs and mice, is one of the most active organs of the body in terms of turnover of zinc On the other hand as indicated earlier the zinc concentration of the pancreas of diabetics is not significantly lower than that of nondiabetics, although the insulin content is markedly lower This cannot be interpreted as evidence that zinc plays no part in insulin production but it strongly suggests that lack of zinc in the diabetic pancreas is not the reason for the reduced insulin production

5 *Minimum Zinc Requirements*

No precise assessment of the minimum requirements of zinc for optimum growth in rats or mice can yet be made The minimum requirement of the growing rat is clearly more than 15 μg Zn per day since growth was severely retarded on diets supplying this amount of zinc daily and very much greater growth was obtained by the addition of liberal amounts of zinc alone to such diets (84) Nutritional studies with still more zinc deficient diets have shown that the growth of rats is proportional to the amount of zinc added up to an intake of 20 μg Zn per day and that more than this amount is needed daily for normal growth (19) How much more can only be determined by further experimentation

VI Zinc in Human Nutrition

Low intakes of zinc have not been associated with any disability in man apart from the suggestion of Eggleton (31) that lack of zinc may be a factor in the beri beri syndrome This suggestion arose from his finding that the diets of the poorer classes of China where beri beri was prevalent supplied only about one half of the amount of zinc obtained from more satisfactory diets and that the blood and epidermal structures of beri beri patients contained much lower concentrations of zinc than those tissues in healthy individuals (Table 25) As far as is known this question has not been investigated further although a possible relationship between zinc and thiamin has been postulated on independent but relatively slender evidence as indicated in the previous section

On the basis of a number of balance experiments but a rather limited consumption range it has been tentatively concluded that an ingestion of 0 3 mg Zn per kg body weight per day will supply the zinc needs of the preschool age child (77) An ingestion of 0 4–0 6 mg per kg body weight per day has been indicated by other workers (83) This corresponds to an intake of 7–14 mg zinc daily for a 50-lb child The average daily intake of this element by adults consuming normal well

balanced diets is about 10–15 mg. An estimate of the daily consumption of zinc from a typical North American diet is 12 mg (32), which may be compared with 9 mg (1) from an average North China diet and 24 mg from the Steffanssen Anderson exclusive meat diet (32).

The actual level of intake from any particular diet will depend principally upon three factors. These are (1) the extent to which it contains meat and other protein sources with which the zinc in foods is particularly associated, (2) the proportion of refined cereals and other carbohydrates compared with whole cereal products, because the zinc is concentrated in the germ and the outer, "branny" layers of the grain, and (3) the degree of contamination of particular foods with zinc from zinc lined pipes and containers. In this connection it should be pointed out that there is no evidence that any nutritional disabilities consequent upon the consumption of diets high in refined carbohydrates are related to the incidental reduction in intakes of zinc which would, of necessity, be imposed. Even patent wheaten flour, representing the lowest extraction cereal material used in quantity in human dietaries, contains appreciable amounts of zinc, probably about 7–8 p p m. Nor, at the other end of the scale, is there any worth while evidence of deleterious effects arising from excessive consumption of zinc due to contamination with this element through corrosion of zinc or zinc lined vessels in processing. Such adventitious sources of zinc may be considerable, especially from milk, but as will be shown later, they are of little practical significance owing to the relatively low toxicity of the element.

Common foods do not readily fall into groups or classes in accordance with their average contents of zinc, as is possible with respect to their iron and copper contents. Analyses by modern accurate methods are relatively scanty and the variability is high. It appears nevertheless that white sugar and pome and citrus fruits are among the lowest in zinc content (usually $<$ 1 p p m of fresh edible portion) and that wheat germ and bran (80–150 p p m), gelatin, and oysters (usually 150–500 p p m and occasionally much higher) are among the richest sources of zinc. Between these extremes, in ascending order of magnitude, occur tubers and root vegetables, white flour and bread, milk, leafy vegetables, meat, fish and eggs, whole cereals, nuts and leguminous seeds, cocoa, and molasses.

VII Absorption and Excretion

The pattern of absorption and excretion of zinc is very similar to that of iron. Studies with several species using stable and radioactive zinc, indicate that this element leaves the body very largely by way of the

feces whether it is ingested or injected (28 57 61 79) When taken in with the food, most of the fecal zinc consists of the unabsorbed portion of the amount ingested A small but significant proportion comprises that fraction which has been absorbed and excreted into the intestine chiefly, it appears, in the pancreatic juice and only in minute amounts in the bile (61) Similarly when injected most of the zinc is excreted in the feces via the pancreatic juice and very little appears in the urine (57 79)

The quantities of zinc excreted in the urine of normal individuals are exceedingly small (0 1–0 9 mg/day), compared with the amounts ingested These quantities of urinary zinc do not vary appreciably with the level of intake in the food and are not significantly increased even when the plasma zinc level is raised following zinc injections (28 57) It is apparent that the kidney has an extremely limited capacity to excrete this element under normal conditions as is the case with iron McCance and Widdowson (57) have in fact suggested that what little zinc does appear in the urine is possibly only an end product of a metabolic function of the kidney itself

The supposition mentioned previously that most or all of the zinc in plasma is bound to protein and for this reason does not readily pass into the urine raises the interesting question of the level of urinary excretion of this metal in individuals with albuminuria It was concluded from an early survey of this subject that the amount of zinc in urine bears no relationship to the *degree* of albuminuria although high contents of zinc are found in the urine of nephritics (34) A more recent study of this problem has disclosed that patients with albuminuria excrete on the average 7 times as much zinc in their urine daily as do normal individuals (57) The actual urinary excretion of 6 such patients was found to range from 1 0 to 3 8 and averaged 2 1 mg Zn per day whereas that of normal individuals varied from 0 11 to 0 50 and averaged 0 3 mg/day The individual amounts could not be correlated however with the degree of albuminuria as was demonstrated in the earlier investigation

Studies with rats and cattle using radioactive zinc have revealed great variability in individual ability to absorb this metal from the intestinal tract together with some evidence of increased absorption in pregnant females during the last third of pregnancy (23) as occurs with iron in pregnant women Interesting and suggestive data pointing to poor absorption of zinc from ordinary diets were obtained in the aforementioned study of albuminuria patients (57) It would be expected that if zinc were readily absorbed and the surplus excreted back

into the gut, less *zinc would be excreted in the feces* of albuminurics with their high urinary excretion, than in the feces of normal subjects It is apparent from the figures of Table 27 that the albuminuria patients excreted in their feces as much zinc as they ingested in addition to the much larger amounts in their urine They were in fact in appreciable negative zinc balance Extrapolation from such limited data is exceedingly dubious as was appreciated by the authors They conclude *nevertheless that zinc is not freely absorbed from ordinary* diets and that patients with albuminuria may not absorb the metal rapidly enough *from ordinary diets to make good* their *urinary loss*

TABLE 27
ZINC BALANCES OF PATIENTS WITH HEAVY ALBUMINURIA (57)

Subject	Intake (mg /day)	Output (mg /day) Urine	Feces	Total	Balance (mg /day)
M E	13 9	3 0	15 3	18 3	–4 4
H E	13 7	2 3	13 8	16 1	–2 4

The similarity between zinc and iron in absorption retention and excretion, mentioned at the commencement of this section is emphasized by the following points both are very poorly absorbed from the gastro intestinal tract both are retained by the body with great tenacity both have an exceedingly small capacity to leave the body by way of the urine and both exist in the body very largely bound to protein There are however, far wider gaps in our knowledge of zinc metabolism than of iron In particular nothing is known of the mechanism of zinc absorption of the main sites of absorption in the alimentary tract *or of the dietary or other factors influencing the degree of absorption* of this metal *from the tract*

VIII Zinc Toxicity

Zinc is relatively nontoxic to mammals Intakes well beyond those which induce symptoms of copper or molybdenum poisoning are tolerated without observable *ill effects Occasional occurrences* of zinc poisoning have been reported in humans as a result of consuming acid foods which were cooked in galvanized iron vessels (60) and in young pigs after being fed *on skim* milk passed through galvanized iron pipes *(41)* The intakes of zinc necessary to induce deleterious effects are however many times those likely to be obtained *from ordinary foods* and beverages thus ensuring a very wide margin of safety for this element *Nutritional interest in* zinc toxicity arises principally from its interaction

with other trace elements notably copper and from the curious fact that certain of the manifestations of zinc toxicity resemble those which supervene as a result of zinc deficiency

Intakes of zinc equivalent to 0 25% or 2500 p p m of the diet are completely without effect on rats over several generations, whether the zinc is ingested as the metal the chloride or the carbonate (44) At double these intakes of zinc growth of rats is severely depressed with heavy mortality in young animals when ingested as the chloride and growth is slightly depressed without heavy mortality when ingested at this level as the oxide (44) Intakes of 0 5% or 1 0% zinc as the carbonate produce severe anemia in addition to subnormal growth anorexia and at the higher dose rate a high proportion of deaths within a few weeks (85) These effects of zinc in rats have been confirmed and extended by Smith and Larson (80) who established that "zinc anemia" is of the microcytic hypochromic type and that it is not the cause of the depressed growth This was deduced from their findings that supplements of copper but not of iron or cobalt prevent the anemia that develops on diets containing 0 7% zinc without preventing the growth inhibition whereas supplements of a liver extract produce a marked growth response without significantly improving the blood picture It seems that there are two distinct and apparently unrelated symptoms of zinc toxicity in these species namely anemia and subnormal growth

In the absence of any copper analyses of the blood and tissues of rats fed excess zinc it is impossible to state whether the zinc anemia is caused by depression of copper assimilation from the basal diet so that the copper concentrations within the tissues are below the levels necessary to sustain normal hematopoiesis or whether the zinc interferes with the availability of the copper after it has reached the tissues as can occur with molybdenum There seems no doubt however that high levels of dietary zinc can induce the copper deficiency state within the rat and that zinc anemia is the result of such an induced deficiency Uncomplicated copper deficiency in the rat is known to be accompanied by subnormal liver cytochrome oxidase and catalase activities which can be restored by copper supplements (73 74) A similar marked reduction in liver cytochrome oxidase and catalase activities has been demonstrated in rats fed diets normally adequate in copper but containing 0 5–0 7% zinc (91) These activities can be maintained at normal levels by copper supplements (0 2 or 0 4 mg Cu per day as copper sulfate) (91) as is shown in Table 28 Since the reduced activities of the enzymes are apparently not mediated by the formation of enzyme inhibitors nor by direct inhibition by zinc ions nor by reduced copper

intake due to the lowered food consumption of the high zinc diets (91) it is safe to assume that the zinc antagonizes either the absorption of copper from the gastrointestinal tract or the utilization of copper within the tissues, or both In any case it is clear that, under conditions of zinc excess, there is a breakdown in the mechanism of hematopoiesis and of formation of active cytochrome oxidase and catalase for both of which adequate concentrations of available copper are necessary

TABLE 28
THE INFLUENCE OF ADDED DIETARY COPPER ON ZINC TOXICITY IN RATS[a] (91)

Dietary conditions	Liver cytochrome oxidase Δ O D per mg protein	Liver catalase moles perborate per mg protein
Basal diet	1 65 ±0 14	5 9 ± 0 2
Basal diet + 0 5% Zn	0 66 ± 0 09	3 3 ± 0 7
Basal diet + 0 5% Zn + 0 2 mg Cu per day	1 58 ± 0 21	6 3 ± 1 0
Basal diet + 0 5% Zn + 0 4 mg Cu per day	1 70 ± 0 10	6 4 ± 1 0

[a] Mean values of 4 determinations

From the poor growth of rats on high zinc diets, and the failure of copper supplements to overcome this particular effect it seems that zinc influences metabolic processes other than those involving copper A series of studies by Sadasivan (71) has revealed the extent of the metabolic changes which can occur in zinc toxicity Zinc oxide at levels of 0 5% and 1 0% of the diet was found to cause a significant lowering of the fat content of the livers of rats on a basal diet upon which control rats developed fatty livers This was interpreted as evidence of a lipotropic action of zinc at these concentrations Development and mineralization of the bones were also adversely affected on these high zinc diets in addition to the marked depression of growth observed by other workers Subsequent investigation disclosed that toxic levels of zinc reduce the assimilation of several of the food constituents in much the same way as is claimed to occur under conditions of zinc deficiency Sadasivan demonstrated that fecal excretion of nitrogen phosphorus and sulfur increases urinary excretion of nitrogen uric acid and creatinine increases and urinary excretion of phosphorus and sulfur decreases on the high zinc diets In further studies this worker showed that the decreased assimilation of phosphorus from the intestine (believed to be the cause of the poor mineralization of bone) is not due to its precipitation as insoluble zinc phosphate and is associated at the 1% level of dietary

zinc with a significant decrease in intestinal phosphatase activity a
a significant increase in liver and kidney phosphatase activity V
Reen (91) also observed that the alkaline phosphatase activity of t
livers of rats consuming diets containing 0 7% zinc as zinc carbona
was 2 to 3 times the normal value

An obvious conclusion from the various studies of zinc toxicity whi
have been described is that this element affects a wide range
metabolic processes in the cells and tissues of the body An essentia
similar conclusion was reached from a consideration of the effects
zinc deficiency It is easy to press the similarity between zinc deficien
and zinc excess too far but it is surely not coincidence that in bo
conditions growth of rats is seriously retarded to an extent grea
than that which would be expected from the lowered food intake fo
assimilation is impaired catalase activity of the liver is reduced a
the alkaline phosphatase activity of the tissues is disturbed Many qu
tions however remain unanswered It is not known for instan
if there is a *reciprocal* antagonism between zinc and copper as occ
with molybdenum and copper If so then high intakes of copper shou
accentuate zinc deficiency The relation of zinc toxicity to molybden
toxicity effects on growth and on copper metabolism is also worthy
further investigation On present evidence it appears that they are u
related processes Additions of zinc have been shown to accentuate t
growth inhibiting effect of high molybdenum diets with rats (40) a
methionine supplements at levels which prevent molybdenum toxic
effects in this species have been reported not to counteract toxic lev
of zinc but actually to cause additional growth retardation (15)

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CHAPTER 8

MANGANESE

I Historical Background

The first demonstration of the presence of manganese in biological material is credited to Scheele (63), who detected this element in plants as early as 1785 but interest in a biological role for manganese did not begin until 1897 when Bertrand (8) formulated his theory, now disproved, that this metal is the active constituent of the oxidases Bertrand and Medigreceanu (9) were among the earliest workers to show that manganese occurs constantly in the tissues and organs of plants and animals including man and within a few years it was clearly established that manganese in contrast to iron occurs in plant tissues in much higher concentrations than in animal tissues It was shown further that it tends to accumulate in the reproductive organs and that the concentrations in the tissues of mammals are relatively constant (11 46 57 59 67)

The invariable presence of manganese in animal tissues in relatively constant proportions led during the 1920s to a number of attempts to determine an essential function for this element in the animal organism (10 45 50 51) These investigations provided suggestive but not conclusive evidence that manganese is an essential element for growth in small laboratory animals As with most of the early efforts to demonstrate an essential role for one or another of the trace elements the diets employed were so deficient in other essential nutrients that even with added manganese the animals made little growth or survived for only short periods In 1931 unequivocal evidence was obtained by Kemmerer Elvehjem and Hart (41) that manganese is necessary in the diet of mice for growth and the maintenance of normal ovarian activity In the same year Waddell Steenbock and Hart (75) demonstrated that manganese greatly improves the ovulation rhythm in female rats on a milk iron and copper diet and Orent and McCollum (55) obtained results indicating that this metal is necessary in the diet of male rats to prevent testicular degeneration and of females for the proper development and functioning of mammary tissue During the following decade diets were devised lower in manganese content than the mineralized milk diet used in the early Wisconsin studies with which it was shown that manganese is an essential component of the diet of rabbits and

chicks, as well as of rats and mice and that it is required for proper bone development in these species in addition to growth and normal reproduction

Interest in the nutritional significance of manganese was further stimulated by the discovery in 1936 and 1937, of its relation to certain naturally occurring disorders of poultry known as perosis or slipped tendon (79) and nutritional chondrodystrophy" (49) These conditions to be described later were found to be caused primarily by low intakes of manganese from certain diets and to be prevented by adequate supplementation of the diets with manganese salts The severity of the bone disorders characteristic of perosis and chondrodystrophy led to investigations of the relation of manganese to bone formation in mammals and to studies of phosphatase activity of the blood and tissues of manganese deficient rats and birds The early association of perosis with unusually high calcium and phosphorus intakes led also to investigations of the influence of these and other elements on manganese absorption and retention

While these researches were proceeding several enzymes were shown to be activated by manganese *in vitro* including bone phosphatase and intestinal peptidases and one of them liver arginase was claimed to contain this metal as part of its prosthetic group (20) This claim has been supported by very recent evidence indicating that manganese is an essential component of arginase (3) Unequivocal evidence that manganese exists as an integral part of the molecule of other enzymes has not yet been produced but the blood pigment (pinna globin) of one species of shellfish *Pinna squamosa* was shown to contain manganese in place of the usual iron or copper (32) This curious phenomenon is comparable with other isolated experiments on the part of nature such as the occurrence of the zinc containing respiratory pigment in the blood of the snail *Sycotypus* and the vanadium containing respiratory pigment in the blood of a group of marine worms *Ascidia*

As occurred with copper and zinc studies of the metabolic significance of manganese in the higher animals were accompanied by concurrent investigations of its role in plant life Strong indications of its necessity for various metabolic processes in the higher plants were obtained by Bertrand and co workers early in the century but conclusive evidence of its essentiality for growth in such plants was not forthcoming until 1923 (52) This finding was subsequently confirmed by many workers for a wide range of crop and pasture species and a number of naturally occurring diseases of these plants was shown to be due to a lack of available or more properly "readily reducible manganese in certain

soils particularly those more alkaline than about pH 6 7 (44) The use of manganese containing fertilizers or sprays was found to be highly effective in remedying the soil deficiencies and in inducing increased growth and improved health of various plants growing upon the deficient soils

II Manganese in Animal Tissues and Secretions

1 *General Distribution*

All animal tissues so far examined contain manganese in very low concentrations In most tissues the levels are substantially lower than those of copper and the variability for any particular organ or tissue, both within and between species is unusually small The highest concentrations occur normally in the bones liver kidney pancreas and pituitary gland (2–4 p p m on the fresh basis) and similar concentrations have been observed also in the pineal gland and the *lactating* mammary gland of the rabbit (24) The spleen heart lungs muscles brain ovaries testes adrenals thyroid and thymus usually contain less than 1 p p m on the fresh basis The results of Fore and Morton (24) who studied the complete distribution of manganese in the tissues of the adult rabbit by means of an exceedingly sensitive catalytic periodate method (24) together with average figures culled from the literature by these authors for the rabbit and a wide range of animal species are given in Table 29

Very high concentrations of manganese do not occur in mammalian eye tissues associated with the pigmented portions as is the case with zinc and copper but somewhat higher levels are present in the retina aqueous humor and possibly the conjunctiva than in most body tissues (24 70) For ox retina a mean value of 1 1 p p m of dried tissue was found by Fore and Morton (24) by means of their sensitive catalytic method mentioned earlier and 2 6 p p m by Tauber and Krause (70) who used the ordinary periodate method Most of the manganese in the retina is nondialyzable and therefore probably bound to protein but whether it serves any particular function in this site is unknown The rods the photoreceptors of scotopic vision appear to contain smaller concentrations of this metal than the remainder of the retina which can be interpreted as evidence against manganese in the retina being selectively or specifically concerned in the visual process (24)

The relative constancy of the manganese levels in the tissues would suggest that the amounts and concentrations present are not greatly influenced by differing dietary intakes This is probably true for a large proportion of the body tissues as is the case with many trace

elements There is evidence from several species however that the levels of manganese in the liver and bones are reduced below normal by subnormal intakes and increased to moderately high levels by high intakes of manganese This has been demonstrated for the rat (47),

TABLE 29
CONCENTRATIONS[a] OF MANGANESE IN ANIMAL TISSUES

Tissue	Rabbit[b]	Rabbit average figures recorded in the literature[c]	Average figures recorded in the literature for a wide range of animal species[c]
Bone (long)	—	3 5	3 3
Pituitary	2 4	—	2 5
Liver	2.1	1 9	2 5
Pancreas	1 6	2 3	1 9
Salivary gland	1 4	—	1 1
Kidney	1 2	1 1	1 2
Duodenum	1 1	—	1 9
Stomach	1 0	0 90	0 80
Hair	0 99	0 90	0 80
Gall bladder	0 91	1 0	1 1
Caecum	0 82	0 43	0 43
Adrenals	0 67	—	0 40
Ovaries	0 60	—	0 55
Bile	0 48	—	0 90
Thymus	0 45	—	1 6
Hide	0 38	0 20	0 40
Testes	0 36	0 40	0 50
Brain	0 36	0 60	0 40
Heart	0 28	0 21	0 34
Spleen	0 22	0 55	0 40
Muscle	0 13	0 14	0 18
Thyroid	0 24	—	0 55

[a] Measured in p p m Mn on the fresh basis
[b] Fore and Morton (24)
[c] Taken from the table of figures presented by Fore and Morton (24)

rabbit (21), pig (38) and chick (36) It has been shown further that the manganese concentration in the ovaries of cattle is greatly reduced under conditions of low manganese intake Supporting data for these statements are given in the following sections

2 *Manganese in Bone*

A good deal of attention has been devoted to the manganese content of bone because of the relation of this element to bone formation Many

of the values reported are so discordant that they throw considerable doubt on the reliability of some of the methods of estimation which have been employed Thus Kehoe and co workers (39) report values of 1 7 p p m and 3 0 p p m for normal fresh human bones whereas Fore and Morton (24) obtained values ranging from 0 27 to 0 51 p p m for fresh ox femur The higher figures are for compact and the lower for cancellous bone Normal rat and chick bones have been found to contain 2 2 and 2 0 p p m respectively *on the dry basis* compared with 0 8 and 0 6 p p m on the same basis for these species following the consumption of manganese deficient diets (26 47) Mean levels of 0 6 and 0 44 p p m of dry fat free bone (femur) have similarly been found in rabbits subsisting on a severely manganese deficient diet compared with 3 1, 5 7 and 12 9 p p m for the femurs of similar rabbits receiving the same diet supplemented with 1 2 and 4 mg manganese as manganese chloride daily (68) From the results of a second experiment carried out by these investigators designed to determine more precisely the minimum manganese requirements of rabbits it would seem that between 2 and 3 p p m represents a "normal level of manganese in the dry fat free bone of this species which is closely similar to the values reported for the bones of normal rats and chicks

It is apparent from the figures quoted above and from the weight of bone in the body that a high proportion of the total body manganese must be situated in the skeleton and that this constitutes a significant "store of this element The concentration of manganese does not reach a maximum level so readily in the bones as it does in the liver

3 *Manganese in the Liver*

The concentration of manganese in the liver varies very little with the species or with the age of the animal No reserve store of this element is provided in the newborn rat rabbit guinea pig pig or man (14 48 64) Human livers from individuals of all ages have been found to be extremely constant in manganese content (6–8 p p m dry tissue) The levels in the livers of sheep and cattle are very similar and also show small individual variability at least by comparison with the widely fluctuating levels of liver iron and copper in these species Average normal values found for adult sheep (72) cattle (29) and hens (62) are in each case 8–10 p p m Slightly lower average values have been reported for the livers of the rat rabbit and guinea pig (48) In these latter species the concentration in the liver rises slightly but significantly between birth and weaning As soon as solid food begins to be consumed in quantity the amounts but not the concentrations of manganese increase substantially (48)

The absence of reserve stores of manganese in the liver of the newborn mammal is in contrast to the position with iron and copper, but is similar to that of zinc This is perhaps surprising when it is considered that milk is relatively almost as low in manganese as it is in iron and copper In fact, milk has been employed as the principal dietary item in practically all diets used for the production of manganese deficiency in mammals It was pointed out in the appropriate sections dealing with iron and copper that the liver stores of these elements in the newborn make a very small contribution to the needs of the suckling Nevertheless, they do make a contribution which is denied to the suckling in the case of manganese

It appears that the liver has only a limited capacity to store manganese at any age and that the only substantial body store of this element resides in the bones Raising the manganese intakes of pigs from a base level of 12 p p m to the equivalent of 172 p p m only increased the mean concentration of manganese in the livers of these animals from 8 8 to 10 3 p p m on the dry basis Larger increases than these were obtained in the livers of rabbits fed various levels of manganese (Table 30) but the greatest increases observed are modest by comparison with

TABLE 30
MANGANESE CONTENT OF THE LIVERS OF RABBITS (68)

Mn fed (mg /day)	Number of animals	Average Mn content of livers (p p m dry tissue)
Basal diet[a]	10	0 9
Basal diet + 0 3	8	6 8
Basal diet + 0 6	7	9 8
Basal diet + 1 0	10	16 2
Basal diet + 2 0	9	20 5
Basal diet + 4 0	9	22 7
Basal diet + 8 0	4	15 4

[a] A fortified milk diet extremely deficient in manganese (0 14 p p m)

those which can be obtained from high intakes of copper and the increases were not significant beyond an intake level of 1 mg Mn per rabbit per day

4 *Manganese in Milk*

Normal cows milk contains 0 02–0 03 mg Mn per liter (2 60) and ewes and mares milk have been reported to contain an average of 0 04 and 0 05 mg /l respectively (60) Data are too limited to permit any

positive statements about the influence of species or of stage of lactation except that cow s colostrum is several times richer in this element than normal milk (60) The normal level can be substantially increased by feeding additional manganese to the cow (2) but there is no evidence that subnormal intakes result in subnormal concentrations in the milk as occurs with subnormal intakes of copper The normal level of manganese in cow s milk is greater than that of cobalt or of iodine but is very much smaller than that of zinc iron, or copper, as shown in Table 26 in Chapter 7

5 *Manganese in Blood*

Data on the manganese content of blood are exceedingly meager largely because the concentrations are normally so low that accurate estimation by existing methods is very difficult According to Kehoe and co workers (39) whole human blood contains 0 12–0 18 μg Mn per ml about two thirds of which is present in the corpuscles Much lower levels than these appear to be common in bovine blood (7 12) but further investigation is necessary before normal values can be established for this or other species or before the relation of blood manganese to varying intakes of this element can be determined

Extraordinary increases in the serum manganese levels of cows turned out to pasture in certain "lactation tetany areas in England have been reported (12) Twenty days after the commencement of grazing on these pastures the serum manganese of six cows was found to rise from the very low mean of 0 01 μg /ml to the exceedingly high mean of 1 5 μg /ml This increase was associated with abnormally high manganese concentrations in the pastures (540–1320 p p m on the dry basis) and with a significant depression of serum magnesium in the animals No such remarkable rise in serum manganese has been reported from elsewhere but a similar depression in serum magnesium has been found in cows receiving a normal ration supplemented with 100 p p m of manganese as manganese sulfate (23) Even in this case supplements of 150 and 200 p p m were not accompanied by a fall in serum magnesium

6 *Manganese in the Avian Egg*

It is pertinent to consider the manganese content of hens eggs because under certain dietary conditions the egg contains insufficient manganese to allow normal hatchability and development of the chick embryo On average rations hens produce eggs containing a total of between 0 01 and 0 02 mg manganese but both the total amount and the concentration are markedly affected by the manganese intake of the

hen Thus Gallup and Norris (27) were able to increase the amount of manganese in the yolk from 0 004 mg to 0 033 mg by raising the manganese content of the diet of the hens from the deficiency level of 13 p p m to the excessively high level of 1000 p p m, and Lyons and Insko (49) found an average of 0 5 p p m on the dry matter of whole eggs from hens receiving a highly manganese deficient ration whereas an average concentration of 0 9 p p m was found in the eggs when this ration was made just adequate by the addition of 40 p p m manganese These findings provide the only certain evidence that a deficiency of a mineral element in the diet of the hen is reflected in a restriction in the amount of the element in the yolk of the egg

As is the case with zinc, copper and iron, the shell is virtually free from manganese but unlike zinc and iron appreciable amounts of manganese occur in the white The *concentration* of manganese in the yolk however is of the order of 4 to 5 times that of the white

7 *Forms of Manganese in the Tissues*

Extremely little is yet known of the chemical forms in which manganese occurs in the body tissues and fluids, or of the extent to which in conformity with iron copper, and zinc it exists in combination with protein Evidence has been presented that almost all the manganese in the mammalian liver is present in the arginase extract (20), indicating that in this organ manganese occurs largely in combination with protein Fore and Morton (24) on the other hand showed by dialysis of liver and kidney homogenates that appreciable amounts of the manganese in these tissues are loosely held, although some is very firmly bound They suggest that in tissues with a relatively high manganese content a portion of the manganese is dialyzable and that there is a very narrow range for the "bound manganese of different tissues It is further implied that some of the manganese in bone and bone marrow is attached to the cell membranes (24) and that this element may be preferentially attached to the cell wall protein throughout the tissues This interesting concept is not entirely new as was pointed out by these authors It has been suggested that oxine inhibits bacterial growth by combining with essential metals in the bacterial surface (1) and been shown that oxine inhibits glutamic acid assimilation in bacteria by combination with a metal in the cell, probably manganese (25)

III Manganese in Mammalian Nutrition

1 *Mice Rats and Rabbits*

Since the original demonstration in 1931 of manganese deficiency in mice and rats (41 53 75) diets have been devised lower in manganese content and better supplied with other essential nutrients The use of these diets most of which contain milk as a major component, has enabled the symptoms of deficiency to be clearly recognized in these species and in rabbits (69), and thus allowed various studies to be undertaken which have thrown some light on the mode of action of this element within the tissues

a. *Minimum Requirements* The minimum requirement of any species for any particular nutrient depends upon the criteria of adequacy which are employed and the nature of the rest of the diet The outstanding significance of these factors in relation to copper has been stressed in Chapter 3 They are also of some importance with manganese because the minimum dietary intakes compatible with maximum growth normal mineralization of bone and normal functioning of the reproductive processes do not appear to be identical Moreover the levels of calcium and phosphorus particularly the latter in the diet are known to affect manganese absorption and therefore dietary requirements

Young mice rats and rabbits are unable to grow normally on milk diets containing 0 1–0 2 p p m Mn and on synthetic diets containing 0 2–0 3 p p m Mn or less unless additional manganese is provided (65 69 74) Their minimum requirement is therefore greater than 0 3 p p m but how much greater is unknown Rabbits fed dried milk (0 14 p p m Mn) plus highly purified iron and copper supplemented with various levels of manganese as manganese chloride develop normal bones and remain free from other signs of manganese deficiency when 0 3 mg Mn per rabbit per day is given but a larger supplement probably between 1 and 4 mg /day appears to be necessary for maximum growth (68) These relatively limited findings apply only to diets well balanced with respect to the amounts and proportions of calcium phosphorus and iron present Lowering the Ca–P ratio from 1 0 85 to 1 2 55 has been found to accentuate the deficiency symptoms in rats consuming a series of diets supplying only 0 5 μg Mn per rat per day (74) This indicates that diets containing an excess of phosphorus in relation to the calcium present impose a higher manganese requirement on the animal

b *Symptoms of Deficiency* The symptoms of manganese deficiency displayed by rats mice and rabbits are similar but not identical Their

nature and severity depend partly upon the species but they are also influenced by the degree of deficiency and the speed with which it is attained and by the duration of the deficiency state These in turn are influenced by the previous nutritional history of the experimental animals, especially their body manganese status There is little doubt that most of the apparently conflicting results on the effects of manga nese deficiency can be explained by variations in one or more of these factors

Manganese deficiency in these species is characterized by impaired growth defective mineralization and structure of the bones and de pressed reproductive function in both males and females Hemoglobin formation is not significantly affected (69 74) but there is some slight evidence of nervous symptoms in very young manganese deficient rats accompanied by incoordination and paralysis (65) These symptoms are associated with lowered arginase activity in the liver and alkaline phos phatase activity in the bones, but such enzymatic changes appear hardly sufficient to account for the deficiency syndrome Similarly the sub normal growth of manganese deficient rats is associated with a lowered food consumption and a lowered efficiency of utilization of the ingested food (13) but whether these alone are sufficient to explain the poor growth or whether more subtle metabolic defects must be looked for as in zinc deficiency remains to be determined

c *Bone Formation* Manganese deficient mice rats and rabbits in variably reveal poorly mineralized bones In rabbits and occasionally in rats this is accompanied by gross bone deformities Thus abnormal tibias (4) and a shortening and bowing of the forelegs (65) have been observed in some manganese deficient rats and severely deformed front legs in a very high proportion of manganese deficient rabbits (69) The appearance of the animal showing this gross deformity of the front legs is shown in Fig 19 The length weight density breaking strength and ash content of the long bones are significantly reduced but not the volume of the bone or the composition of the bone ash other than a reduction in its manganese content (Table 31)

Microscopic examination of the humeri of the manganese deficient rabbits for which the gross data are given in Table 31 revealed ex tensive deviations from the normal which differ distinctly from the changes found in rachitic bones The most evident of the deviations from normal is a narrowing of the zone of provisional calcification due to an appreciable reduction in the number of cartilage spicules As a result of the reduction of cartilage spicules the epiphyseal plate is narrow and sharply defined and there is a deficiency of spongy bone

There is no evidence of accelerated bone resorption The over a is interpreted by the authors as a suppression of osteogenesis (

The bone disorder of manganese deficiency in rabbits not on distinctly from rickets it also differs distinctly from that o deficiency in dogs described in Chapter 3 The two abnormalit

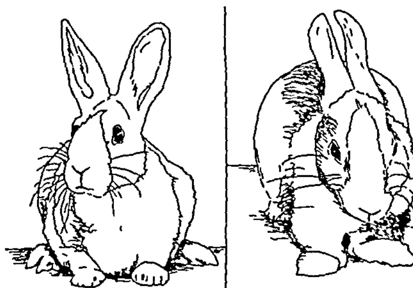


Fig 19 Normal rabbit (left) and Mn deficient rabbit showing deformity of the front legs (right) (Smith *et al* 69 redrawn by J Car

a superficial similarity which can be seen by comparing the ap of the legs of the manganese deficient rabbit in Fig 19 with the copper deficient dogs in Fig 8 but the latter condition is terized by excessive absorption of bone and consists of a diffu porosis with no primary disturbance of the calcification me whereas manganese deficiency clearly involves an impairmen calcification process without any evidence of accelerated bon

TABLE 31

THE EFFECT OF MANGANESE DEFICIENCY ON THE LONG BONES OF RABBITS (69)

	Basal Mn deficient diet + Mn[a]	Basal Mn deficient diet[b]	Significance
Weight of dry fat free humeri (g.)	1.205 ± 0.0411	0.923 ± 0.102	significant
Volume of humeri (ml.)	1.587 ± 0.0732	1.449 ± 0.1192	none
Density of humeri (g./ml.)	1.040 ± 0.0151	0.818 ± 0.0562	highly significant
Length of humeri (mm.)	59.70 ± 0.853	52.63 ± 2.113	highly significant
Diameter of humeri shaft (mm.)	3.78 ± 0.062	3.79 ± 0.129	none
Breaking strength of ulnae (lb.)	13.54 ± 0.994	9.04 ± 1.490	significant
Ash in dry fat free humeri (%)	61.38 ± 0.469	55.67 ± 0.981	highly significant

[a] Mean values of 6 animals

[b] Mean values of 7 animals

alkaline phosphatase activities of the ulnae manganese deficient 9 3 units/g and 5 4 units/g fresh ends of bones and whole bones respectively manganese fed (1 mg /day) 32 5 units/g and 9 2 units/g of fresh ends of bones and whole bones respectively It appears that manganese affects bone formation at least partly through its influence on bone phosphatase activity

d *Reproduction* Defective ovulation in female rats testicular degeneration and sterility in male rats and early mortality in young rats were among the earliest observations of the effects of manganese deficiency Claims that deficient lactation was the cause of the infant mortality were later elegantly disproved by placing the young from normal rats with manganese deficient lactating mothers (19) These young developed normally The fact that manganese deficient females show no loss of ability to suckle normal young and no lack of maternal interest in their young has been confirmed (65) Shils and McCollum suggest that there are three stages of manganese deficiency in the female In the least severe stage the animals give birth to viable young which develop symptoms of incoordination and even paralysis In the second and more severe stage nonviable young are born which die shortly after birth In the third and most severe stage of deficiency estrous cycles are either absent or very irregular the animals will not mate and sterility results These last mentioned effects were produced within a few weeks in young female rats on a highly manganese deficient milk diet and in addition a marked delay in the opening of the vaginal orifice was observed (13)

No explanation of these profound effects of manganese deficiency in female rats has yet been produced Histological examination of the ovaries has revealed no significant abnormalities in either rats or rabbits Of possible significance is the marked reduction in manganese concentration in the ovaries of cattle that has been observed on low manganese rations even when other tissues examined revealed concentrations within the normal range (7) In the manganese deficient male rat and rabbit the sterility and absence of libido by contrast are associated with marked testicular degeneration Extensive tubular degeneration complete lack of spermatids and spermatozoa and accumulation of degenerating cells in the lumina of the epididymis have been demonstrated (13 69) The underlying mechanisms involving manganese which are responsible for these changes are completely unknown although it is obvious that this element is essential in the process of spermatogenesis

e *Arginase Activity* In the manganese deficient rat and rabbit there is a significant reduction in liver arginase activity and in liver manganese

concentration (13, 65 68), compared with those of healthy animals fed stock diets or the same basal diet plus adequate manganese The reduction in arginase activity may be as much as 50% or more in individual deficient animals and in manganese concentration as much as 90% A feature of the findings with liver arginase is that the activity can be raised substantially by adding manganese to the liver preparations under test Shils and McCollum (65) using arginine carbonate as the substrate were able, by these means to raise the liver arginase activity of the manganese deficient rats to that of the controls whereas Boyer and co workers (13), using arginine hydrochloride as the substrate obtained arginase activity in the control livers plus manganese approximately double that in the manganese deficient livers plus manganese The question as to whether in manganese deficient rats only the activation of the enzyme is reduced or whether the formation and concentration of the protein part of the enzyme are also reduced remains at present unanswered It would be of great interest to determine the arginase activity of liver homogenates from manganese deficient and normal rats with and without *in vitro* additions of elements other than manganese, that have been shown to be effective activators of relatively pure preparations of this enzyme

It can be asked, also, if decreased arginase activity *in vitro* is accompanied by a similar decrease *in vivo* and if so whether the decrease is sufficient to account for any of the symptoms shown by the deficient animal The answer, on the limited evidence available appears to be No In manganese deficient rats the proportion of total urinary nitrogen excreted as urea was found to be only very slightly reduced and the proportions excreted as ammonia, amino acid, and uric acid to be unchanged (13) Furthermore increasing the burden on the urea forming system by feeding ammonium citrate to such animals does not accentuate the deficiency symptoms (65)

2 *Pigs*

Manganese does not appear to be a critical element in practical swine rations even when these rations comprise a high proportion of corn, which as is shown later is normally very much lower in manganese than all other grains A high corn high mineral ration containing 11–14 p p m Mn has been reported to be satisfactory for the growth of pigs but to result in leg stiffness and lameness which can be prevented but not cured by additional manganese (40) Some slight indications of an improved reproductive performance from additional manganese without significant effect on growth or an observable effect on bone develop

ment have also been recorded in pigs confined on concrete and receiving a high corn diet containing about 12 p p m Mn (33) These findings suggest but do not prove that a dietary manganese intake of approximately 12 p p m might be regarded as marginal for completely satisfactory nutrition of pigs Most practical rations would supply at least twice this level of manganese

On the other hand Johnson (38) has obtained evidence pointing to a very much lower requirement for this species at least for growth This worker fed a special diet based on dried skim milk which contained less than 0 5 p p m Mn On this ration young pigs made entirely satisfactory growth and no bone abnormalities or difficulties in leg movements were observed Reproduction was poor however, and udder development inferior Both these conditions were satisfactory when the low manganese diet was supplemented with a green feed supplement that raised the manganese content of the whole ration to 6 p p m but it is not clear whether the manganese was responsible for the improvement or other factors in the green feed It is obvious that the whole question of manganese in swine nutrition warrants further investigation

3 *Cattle*

Under natural conditions of grazing or stall feeding manganese deficiency in cattle (or in sheep goats or horses) is likely to be extremely rare because of the normally high manganese content of fodders Most pastures and hays contain 50–150 p p m Mn on the dry basis and seeds and seed products (corn is a notable exception) 15–50 p p m (6 62) Very many samples have been shown to contain much higher concentrations than these Some of the factors influencing the manganese content of such feed stuffs are considered below but it is apparent that the consumption of rations composed of these materials would normally supply many times the minimum levels of intakes required by small laboratory animals and pigs

Suggestive evidence that approximately 10 p p m Mn is definitely borderline or marginal for optimum reproductive performance in dairy cows although sufficient for growth in heifers has been presented by Bentley and Phillips (7) These workers found a number of samples of hay used in their experiments to be exceptionally low in manganese Many contained less than 20 p p m and some samples less than 10 p p m Mn On rations including such low manganese legume hay plus corn silage and a grain mixture of corn and corn products which contained an over all manganese content of 7–10 p p m in the dry matter it was observed that heifers grew just as well and cows produced as much milk

as when these rations were supplemented with 30 40 or 60 p p m manganese They were, however, slower to exhibit oestrus were slightly and consistently slower to conceive, and a greater number of calves were born with weak legs and pasterns at the first calving The ovaries of the cattle consuming the unsupplemented ration averaged only 0 85 p p m Mn of the dry tissue, compared with 2 0 p p m for the ovaries of the animals receiving this ration plus 30 p p m manganese There was no significant difference between the manganese concentration in the livers and other tissues examined from the supplemented and un supplemented cows The authors suggest that cattle rations should con tain not less than 20 p p m Mn to provide an adequate margin of safety

Added significance is given to the pioneer work of Bentley and Phillips and to the need for further investigation of the manganese needs of cattle by a recent report from Holland (31) which indicates for the first time, that manganese deficiency in cattle may occur under natural conditions in the field On certain sand and peat soils in the Netherlands disorders occur in young cattle which suggest manganese deficiency and which respond either to a dietary supplement of 2 g manganese sulfate per head daily or to treatment of the pastures with 15 kg manganese sulfate per hectare (31) The main symptoms are poor growth and body development leg deformities with overknuckling poor fertility fre quent abortion and dead, dry and brownish hair Except in extreme cases spontaneous cure of the leg deformities usually occurs in the adult Manganese analyses of the organs of one affected animal disclosed normal concentrations of this element in the few tissues examined except for the spleen and ovaries The value found for the ovaries (0 6 p p m of dry tissue) may be compared with the value of 0 85 p p m obtained by Bentley and Phillips for their cattle on low manganese rations

Examination of affected soils and pastures revealed that the former are low in exchangeable manganese, high in iron and potassium and with a pH ranging from 6 0 to 6 9 The pastures are not exceptionally low in manganese content Of 21 samples ranging from 44 to 199 p p m on the dry basis, 12 were under 100 p p m The authors consider that the unusually high Ca P Fe, and protein contents of these pastures raise the requirement of the animal for manganese These highly in teresting observations need much further critical study before the condi tion can be established unequivocally as a manganese deficiency but on present evidence it appears very probable The results obtained so far point to the occurrence of a manganese deficiency conditioned by abnormalities in the diet in other respects They provide additional evidence which was particularly stressed in relation to copper and

molybdenum of the necessity of considering the composition of th whole diet when assessing the possibilities of trace mineral deficienci or excesses

4 *Man*

Manganese has not yet been shown to be required by the hum organism although there is every reason to expect that it functio qualitatively for this species as it does for small laboratory animals ar that it is therefore necessary for growth bone formation and reprodu tion The possibility of slipped epiphyses in children being related perosis in chicks has been mooted (71) Otherwise lack of mangane has never been linked with any disability or malady in humans

From the results of balance experiments Everson and Daniels (2 recommend that the diets of children should contain 0 2–0 3 mg M per kg body weight which would amount to 3–5 mg daily for a 35 l child This appears very high and would not always be easy to obta from an ordinary child s diet high in dairy products and white flour A intake of 4 6 mg Mn daily which is one estimate given for maintaini adult males in manganese balance (5) would also not be easy to obta from diets high in refined cereals and dairy products It would how ever readily be obtained from diets containing liberal amounts of gree leafy vegetables and a proportion of unrefined cereals Thus Kent ar McCance (42) found the average daily intake of two adults consur ing a diet in which 40–50% of the calories came from white flour to l 2 2–2 7 mg Mn daily whereas in two adults consuming a diet in whi the same proportion of the calories came from 92% extraction flour whi would include most of the bran and embryo the daily intake of mang nese was 8 5–8 8 mg An average intake of 7 mg Mn per day w calculated for adults on a typical English winter diet (54) but it shou be noted that no less than 3 3 mg of this came from tea which is e ceptionally rich in manganese (150–900 p p m) A considerable propo tion (about one third) of the manganese in tea is soluble in hot wat and is therefore consumed following infusion

No other ordinary item in the diet except blueberries approaches t in manganese content Peterson and Skinner (56) analyzed the edib portion of 138 common foods and listed them in twelve major fo classes in descending order of their average manganese content the *fresh* basis as follows nuts cereals and their products dried legun seeds green leafy vegetables dried fruits roots tubers and stalk fresh fruits including blueberries fresh fruits excluding blueberries no leafy vegetables animal tissues poultry and poultry products dai

products fish and sea foods including oysters or fish and sea foods excluding oysters The actual average concentrations ranged from 20–23 p p m Mn for the first three classes to 0 2–0 5 p p m Mn for the last three classes (Table 32) It must be realized that leafy vegetables rank fourth only because of their high water content as ordinarily consumed

TABLE 32
MANGANESE CONTENT[a] OF GROUPS OF PRINCIPAL FOODSTUFFS (56)

Class of Food	Number of samples	Minimum	Maximum	Average
Nuts	10	6 3	41 7	22 7
Cereals and their products	23	0 5	91 1	20 2
Dried legume seeds	4	10 7	27 7	20 0
Green leafy vegetables	18	0 8	12 6	4 5
Dried fruits	7	1 5	6 7	3 3
Roots tubers and stalks	12	0 4	9 2	2 1
Fresh fruits (including blueberries)	26	0 2	44 4	3 7
Fresh fruits (excluding blueberries)	25	0 2	10 7	2 0
Nonleafy vegetables	5	0 8	2 4	1 5
Animal tissues	13	0 08	3 8	1 0
Poultry and poultry products	6	0 30	1 1	0 5
Dairy products	7	0 03	1 6	0 5
Fish and sea foods (including oysters)	7	0 12	2.2	0 5
Fish and sea foods (excluding oysters)	6	0 12	0 4	0 25

[a] Measured in p p m Mn (fresh edible portion)

They are easily the highest in manganese content as a group if all are expressed on a dry matter basis

A conspicuous feature of the data presented in Table 32 is the great variability within most of the classes of foods This applies especially to the cereals and their products and to the leafy vegetables The variability in the former is due partly to great differences in the normal manganese content of different grains, which is commented upon in detail in the following section dealing with avian nutrition and partly to the inclusion of materials ranging from patent flour to bran The high concentration of manganese in the embryo and outer layers of the grain results in great variation in the concentration of this element in accordance with the proportions of these parts of the grain included in the product This is illustrated in Table 33

The great variability in the manganese content of the leafy vegetables arises as with the cereal grains partly as a result of inherent species differences but it is mainly due to differences in the nature of the soils on which the vegetables are grown and the manurial or liming treatment

given Thus crops grown on acid soils generally contain significantly higher concentrations of manganese than the same crops grown on neutral or alkaline soils or on the same soils heavily limed Treatment with manganese containing fertilizers also induces higher manganese concentrations in the plants especially where the need for such

TABLE 33

MANGANESE CONTENT[a] OF WHEAT PRODUCTS FROM THE SAME LOT OF GRAIN (62)

Bran	119	Red dog flour	35
Brown shorts	101	Low grade flour	5
Grey shorts	63	Whole wheat (av)	31
White shorts	56	Wheat germ (av)	160

[a] Measured in p p m Mn

treatment is indicated by yield responses to manganese consequent upon subnormal soil status in easily reducible manganese

IV Manganese in Avian Nutrition

1 *Perosis in Poultry*

Recognition of the important role of manganese in avian nutrition arose from studies of a disease of poultry known as *perosis* *Perosis is* characterized by gross enlargement and malformation of the tibio metatarsal joint twisting and bending of the distal end of the tibia and of the proximal end of the tarsometatarsus thickening and shortening of the leg bones (Fig 20) and slipping of the gastrocnemius or Achilles tendon from its condyles As this condition develops and becomes progressively more severe the chicks show great reluctance to move about they squat frequently and are later forced to walk upon their hocks Birds crippled in this way soon die The disease was found to occur most frequently in young battery fed birds and to be aggravated by high intakes of calcium and phosphorus Spectrographic examination by Wilgus Norris and Heuser (79) in 1936 of a sample of mono calcium phosphate which possessed a protective rather than an aggravating effect on the incidence of perosis disclosed the fact that it contained appreciable amounts of manganese and traces of iron and aluminum This finding led to trials of the effect of additions of manganese and other minerals to perosis producing diets The addition of 25 p p m to a basal diet already containing 10 p p m Mn was found to prevent the perosis completely (79) A similar but much less effective preventive action was also reported for zinc and aluminum salts

Subsequent investigation by many groups of workers (80) has pro

vided ample confirmation of the original finding that manganese deficiency is primarily responsible for perosis and that it can be prevented by adequate dietary intakes of this element, but the ability of minerals other than manganese, notably zinc and aluminum, to prevent this

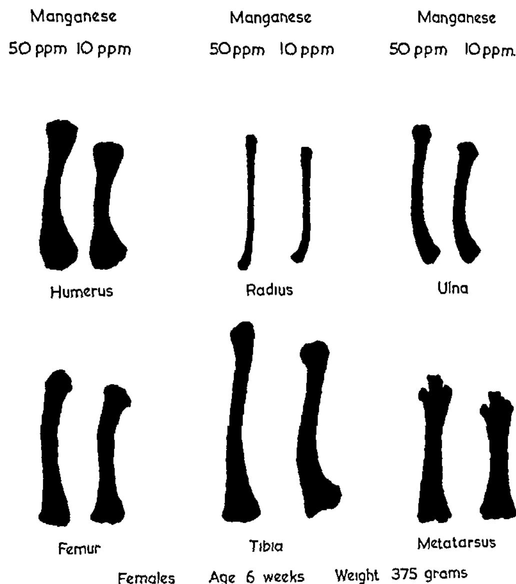


FIG 20 The bones of chicks showing the thickening and shortening which occurs on Mn deficient (10 p p m) rations (Caskey *et al* 17)

condition has not been confirmed (37) However manganese is not necessarily the only factor involved The incidence of perosis may be influenced by the dietary level of several organic nutrients of which choline (35) and inositol (76) appear to be the most important

2 *Chondrodystrophy in Chick Embryos*

In the year following the first demonstration of the relation of manganese to perosis in chicks Lyons and Insko (19) showed that the condition in embryo chicks known as nutritional chondrodystrophy resulted from a manganese deficiency in the diet of the hen Chondrodystrophy varies considerably in degree of expression but the most common characteristics of the embryos are described by Lyons and Insko as "(i) greatly shortened and thickened legs and shortened wings, (ii) parrot beak resulting from a disproportionate shortening of the lower mandible (iii) globular contour of head apparently due to anterior bulging of the skull (iv) edema usually occurring just above the atlas joint of the neck and extending posteriorly for a variable distance, (v) protruding abdomen apparently due to a relatively large amount of unassimilated yolk and (vi) retarded down and body growth particularly in the more severe cases" These characteristics are illustrated in Fig 21

A sporadic type of chondrodystrophy apparently genetic in origin was first reported in 1926 (43) but not until 1935 did Byerly and co workers (15) report the occurrence of a different type of chondrodystrophy in chick embryos and show it to be definitely of nutritional origin In

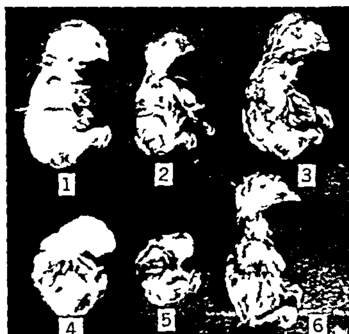


Fig 21 Photos 1 to 4 chondrodystrophic 21-day chick embryos photo 5 19-day chondrodystrophic embryo photo 6 normal 21-day embryo (Lyons and Insko 19)

corporating wheat germ and liver or wheat germ and whey into the ration of the parent stock was found to prevent the disorder These findings, together with those of Wilgus and co workers (79) on perosis led Lyons and Insko (49) to test the effect on the incidence of chon drodystrophy of feeding additional manganese to the hen and of direct injection of manganese sulfate into the egg Supplementing a chondro dystrophy producing diet (5 5 p p m Mn) with 40 p p m manganese as manganese sulfate or injecting 0 03 mg manganese as the sulfate directly into the egg were found to be equally effective in promoting normal hatchability and preventing symptoms of chondrodystrophy It was found, further, that the dietary manganese supplement resulted in the production of eggs of higher manganese content than those in which the embryos developed abnormally and died The marked effect of the manganese content of the hen s diet upon the manganese content of the egg has been referred to earlier More direct evidence of the effect of the hen's diet upon the manganese content of the embryos themselves was then obtained Thirty two chondrodystrophic 20-21 day old embryos were found to contain an average of 2 4 μg Mn per embryo, whereas thirteen 21 day old embryos and three chicks showing normal skeletal development contained an average of 7 0 μg Mn per embryo (49) These findings proved conclusively that this type of chondrodystrophy is due to a deficiency of manganese in the egg consequent upon an inadequate intake from the diet of the hen

3 *Other Effects of Manganese Deficiency in Poultry*

Perosis and chondrodystrophy both of which involve abnormal bone development as the outstanding symptom may be regarded as manifesta tions of severe manganese deficiency The severity of the condition and the proportion of birds or eggs affected will in general depend upon the level of intake of dietary manganese It is clear that where the incidence and severity of these conditions are high the productivity of the flocks will be greatly lowered and the mortality greatly raised Even where perosis and chondrodystrophy are not conspicuous at in takes of manganese which are suboptimal but not acutely deficient the birds are affected in various ways A deficiency of this order has been found to be associated in some birds with nervous symptoms (ataxia) inferior growth of chicks and failure to maintain body weight in mature birds lowered egg production decreased hatchability, and reduced egg shell breaking strength and egg shell ash (62 80) These effects which are accompanied by subnormal concentrations of manganese in the egg embryo and bones and liver of the birds can all be prevented by the

nsion of diets adequate in manganese content and satisfactory in r respects

Minimum Manganese Requirements

he minimum manganese requirements for satisfactory growth health productivity are known more precisely for birds than they are for nmals They depend principally upon the following three factors the breed or strain of the bird (2) the quantity of calcium phos rus and iron in the diet and (3) the source that is the chemical n of the manganese supplied

he lighter breeds have a somewhat lower manganese requirement n the heavier breeds In White Leghorn and New Hampshire chicks p p m and 50 p p m of the diet respectively, have been found just icient for growth and the prevention of perosis (28) Other workers ort a minimum of 35 p p m for their White Leghorns (36) and 40 m for Barred Rocks (62) A slightly higher requirement of this er for Barred Rock hens than for White Leghorns for egg produc hatchability and the prevention of chondrodystrophy has also been nd (30) It is evident from the consistency of these results that the imum manganese requirement in terms of the proportion present he diet is very similar for the growth of chicks and for normal egg duction and hatchability by hens and that an average of 40 p p m is likely to meet the full needs of most birds Individual variability nherent ability to assimilate or utilize manganese is considerable these results apply with certainty only to diets generally based on n (maize) and containing adequate but not excessive amounts of er elements such as calcium phosphorus or iron The influence of h mineral rations and of the considerable variation in the manganese centrations of different feed stuffs are considered below

Excess calcium and phosphorus in the diet increases the manganese uirement of birds by directly affecting its availability In fact the lition of excessive amounts of bone meal or calcium phosphate can ke normal rations perotic In one experiment 64% of the chicks fed ugh mineral ration containing 3 2% Ca 1 6% P and 37 p p m Mn eloped leg bone abnormalities although this level of manganese had en found sufficient to prevent perosis in a high corn ration containing % Ca and 0 9% P Omission of the bone meal from the high mineral ion without additional manganese resulted in freedom from perosis l retaining the bone meal and adding manganese at levels of 62 and 4 p p m reduced the incidence of leg abnormalities to only 5% (62) is effect of calcium phosphate is not due to the removal of manganese

from solution as insoluble hydroxide or phosphate Manganese phosphate is no less available for the prevention of perosis than many other manganese compounds It appears that the mechanism is rather a reduction in soluble manganese through adsorption by solid mineral (61 78) Such adsorption of manganese by carbonates and phosphates especially the latter, has been demonstrated in *in vitro* studies at acid reactions similar to those in the absorptive areas of the intestine (61, 78) The amount of manganese remaining in solution is dependent upon the amount originally present, which makes clear how extra manganese in the diet tends to overcome the effect of excess mineral

All the normal sources of manganese used in the supplementation of poultry rations (oxide carbonate sulfate chloride and permanganate) appear equally valuable (61) Differences in the availability of different chemical forms of this element are therefore of little practical significance Exceptions are provided by one carbonate ore of manganese (rhodochrosite) and a silicate ore (rhodonite), which are relatively unavailable (28, 62)

Two aspects of the manganese requirements of poultry require further comment The first of these is the very much higher requirements of birds than of mammals even under the most favorable conditions with respect to dietary levels of calcium and phosphorus To some extent this may be due to a relatively lower absorption from the gut in the former species, although unequivocal evidence for this is not available Injection of manganese in quantities equivalent to 6–10 p p m of the diet is completely effective in preventing perosis, compared with 4–5 times these quantities necessary in the diet (28 76 78) This suggests a low absorption of manganese—a suggestion that finds support from studies with chicks utilizing radioactive manganese (53) Poor absorption of ingested manganese and practically complete elimination in the feces were observed It is doubtful however if low absorption can completely account for the difference in manganese requirements between birds and mammals especially as the limited evidence indicates that this element is also poorly absorbed in mammals It seems more likely that the former species have also a greater requirement for absorbed manganese although in what way and for what purposes remain to be determined It is perhaps significant in this respect that abnormal bone development is a much more prominent feature of manganese deficiency in birds than in mammals

The relatively high requirement of birds for manganese is the main reason for the great importance of this element in practical poultry raising and for the incidence of naturally occurring deficiencies in this

species whereas it is virtually unknown in other farm stock and is only induced in small laboratory mammals on specially prepared diets An additional factor of great significance is the strikingly low manganese content of corn (maize) compared with all other cereal grains Of the common grains wheat and oats are richest in manganese They contain enough or almost enough to prevent perosis even if the ration is composed very largely of these cereals Barley and rice contain inter mediate amounts of manganese but usually insufficient to meet the need of birds (62) Considerable individual variability exists but the average species differences just mentioned appear to be consistent for widely separated conditions of growth Data from North America (62) and Western Australia (73) are given in Table 34 to illustrate these points

It is apparent from Table 34 that poultry rations based on corn and to a lesser extent on barley are certain to be grossly deficient in manga nese unless supplemented directly with manganese salts or with a high proportion of manganese rich feeds such as wheaten bran or middlings whereas rations based on wheat or oats are likely to provide almost sufficient manganese to meet the birds requirements unless the ration contains excessively large amounts of calcium and phosphorus It is significant that both naturally occurring and experimentally induced perosis and chondrodystrophy have almost invariably developed on high corn diets Nor can protein supplements such as tankage meat meal fish meal or skim milk be expected to assist since they contribute less manganese per unit of ration than do most cereal grains Soybean oil meal an important protein supplement for poultry in some parts of the world averages about 30 p p m manganese (62) Its inclusion would therefore appreciably improve the manganese content of a corn or barley based diet but it would not eliminate the necessity for manganese supplementation

5 *Mode of Action of Manganese in Poultry*

The physiological mechanisms underlying the effect of manganese upon growth egg production and the prevention of nervous symptoms in poultry remain largely obscure but the mode of action of this element in bone formation in these species is better understood

A deficiency of manganese in the diet of chicks results in a significant shortening and thickening of the leg and wing bones as well as a shorten ing of the spinal column (Fig 20) These bone changes cannot be ex plained by an alteration in the gross composition of the body since the gross body composition of both manganese-deficient and normal chicks of the same age is approximately the same (17) The ash content

TABLE 34
THE MANGANESE CONTENT[a] OF CEREAL GRAINS

Source	Corn	Barley	Oats	Wheat	Wheat Bran	Wheat pollard or middlings
North America[b]	5(3.9–11.6)	14(9–19)	36(26–44)	31(24–37)	108(96–120)	101(97–110)
Western Australia[c]	8(6.5–11.6)	15(9–23)	43(27–62)	37(19–84)	133(114–168)	100(62–118)

[a] Measured in p.p.m. Mn on the dry basis
[b] Schaible *et al.* (62)
[c] Underwood *et al.* (73)

of the bones of manganese deficient chicks is also significantly less than that of normal chicks Thus Caskey and co workers (17) report 40 45% ash in the dry fat free bones of 6 weeks old chicks fed a ration containing 5 5 p p m Mn compared with 44 35% for chicks of the same age fed the same diet plus 100 p p m Mn This difference was highly significant but the ash content of the bones of the manganese deficient chicks as has also been shown for manganese deficient rats and rabbits is not nearly as low as can occur in rickets Examination of the bones by X ray and by staining reveals a different picture from typical rickets The serum calcium levels are normal and manganese does not affect bone formation through favorably influencing vitamin D utilization (17) Furthermore the breaking strength of eggs and egg shell ash (CaO) content are directly correlated with the manganese content of the diet of the hens (18) It is apparent therefore that manganese is directly concerned in calcium and phosphorus metabolism in birds

One important means but not necessarily the only means by which this effect of manganese is mediated is by the influence of this element on phosphatase activity Wiese and associates (77) have shown that the blood and bone phosphatase activity of chicks on a perotic diet is very significantly below normal and that the lowering of phosphatase activity *precedes markedly* the appearance of perosis The values obtained by these workers are shown in Table 35

Additions of manganese to phosphatase preparations from the bones of birds with perosis were found to bring about increased activity but failed to activate the enzyme preparation to the level of normal birds (77) This seems to indicate that the *amount* of enzyme present is less in manganese deficient birds and suggests that manganese functions in calcium and phosphorus metabolism in this species as in mammals primarily through facilitating the formation and activity of phosphatase

V Absorption and Excretion

Manganese resembles iron and zinc in that it is poorly absorbed and is excreted largely in the feces In no species has the kidney been found to play an active part in the elimination of this element Most of the manganese in the feces represents manganese which has not been absorbed but part of it consists of metal which has been absorbed and excreted into the intestine via the bile Reiman and Minot (58) found that after patients with biliary fistulae had taken relatively large amounts of manganese by mouth the manganese in the bile might rise to 10 times its former level Studies with radioactive manganese in rats (65) have similarly shown that the liver is particularly active in manganese metabo

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[c] Underwood *et al.* (73)

of the bones of manganese deficient chicks is also significantly less than that of normal chicks. Thus Caskey and co-workers (17) report 40.45% ash in the dry fat free bones of 6 weeks old chicks fed a ration containing 5.5 p.p.m. Mn compared with 44.35% for chicks of the same age fed the same diet plus 100 p.p.m. Mn. This difference was highly significant but the ash content of the bones of the manganese deficient chicks, as has also been shown for manganese deficient rats and rabbits, is not nearly as low as can occur in rickets. Examination of the bones by X-ray and by staining reveals a different picture from typical rickets. The serum calcium levels are normal and manganese does not affect bone formation through favorably influencing vitamin D utilization (17). Furthermore, the breaking strength of eggs and egg shell ash (CaO) content are directly correlated with the manganese content of the diet of the hens (18). It is apparent, therefore, that manganese is directly concerned in calcium and phosphorus metabolism in birds.

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TABLE 35

Blood and Bone Phosphatase Activity of Perotic and Nonperotic Birds (77)

Ration	Perosis	Number of birds	Units of blood phosphatase per 100 ml	Number of birds	Units of bone phosphatase per g green bone
Basal (perosis producing)	Yes	22	8 3(2 1–16 5)	38	4 7(2 3– 7 5)
Basal + 50 p p m Mn	No	21	22 6(19 6–28 0)	39	9 1(7 1–15 8)
Basal + Mg[a]	Yes	6	13 1	6	4 9

[a] 3 mg Mg per week by injection

lism and that as much as 50–75% of the intestinal excretion of such labeled manganese may come from the bile

On the other hand Kent and McCance (42) obtained evidence that manganese when injected in moderate amounts is not excreted at all freely by the human bowel Two individuals injected in this way with manganese butyrate retained the whole of the injected manganese and a third excreted about 50% in the feces In neither case was there any significant increase in urinary manganese following injection It was shown further that raising the dietary intake of manganese approximately four fold by substituting virtually wholemeal flour for white flour produced a very slight increase in manganese retention but most of the extra manganese appeared in the feces with no increase in urinary manganese output The actual excretion in the urine in most cases was only 0 04–0 07 mg Mn daily It is apparent that the absorption of this element from the food is poor and that the body has a negligible capacity to excrete it in the urine It would be of great interest to examine manganese excretion in nephritic patients Iron copper and zinc excretion in such patients is much higher than normal owing presumably to the binding of these elements by protein components of the plasma Whether manganese is similarly bound in the plasma is unknown

VI Manganese Toxicity

All animal species with the possible exception of rabbits exhibit a high tolerance to soluble bivalent manganese salts As much as 3 5 g of manganese citrate daily has been fed to pigs for 9 months without any toxic symptoms appearing (59) 1000–2000 p p m (0 1–0 2%) of manganese in the diet of rats have no effect on their growth although larger amounts interfere with phosphorus retention and hens tolerate 1000 p p m or more without ill effects (28) A ration containing 4800 p p m (0 48%) has been shown to be highly toxic to young chicks (34) These findings indicate the considerable margin of safety which exists between levels of manganese likely to be ingested with the food or used in the prevention of manganese deficiency in poultry and those which induce toxic symptoms

The possible relation between high manganese intakes from certain pastures with abnormally high concentrations of manganese (average 734 p p m compared with a "normal" average of 60 p p m) to "lactation tetany in cows (12) was quoted earlier In addition it has been suggested that susceptibility to the virus of infectious anemia in horses grazing certain pastures in Sweden is associated with the unusually high manganese content of these pastures (16) In neither of these cases has

the association with excessive intakes of manganese been supported b any published findings from elsewhere

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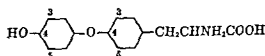
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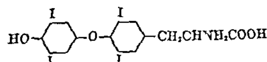
by Coindet, also fell into disrepute partly because the large doses generally employed sometimes induced toxic symptoms and partly because the rationale of iodine therapy was not then understood

A much firmer connection between iodine and thyroid function was established by observations along entirely different lines during the second half of the nineteenth century, when such characteristics of endemic goiter as cretinism myxedema and "cachexia strumipriva" were linked with an absence of thyroid function and with experimental athyreosis The symptoms of myxedema were shown in 1891 to be ameliorated by subcutaneous injections of thyroid gland extracts (see Harington 43) A further substantial step forward was made in 1895 when Baumann (10) demonstrated that iodine is a normal constituent of the body tissues and especially of the thyroid gland, and that the amount of this element in the thyroid is diminished in endemic goiter By the end of the century Oswald (81) had confirmed and extended the findings of Baumann and had identified thyroglobulin

Attention was then turned to the nature of the active components of the thyroid gland culminating in the isolation by Kendall in 1919 (56) of a crystalline compound containing 65% iodine This he claimed was the active principle, which he named thyroxine The structure of thyroxine was brilliantly revealed by Harington in 1926 and in the following year Harington and Barger (45) synthesized this substance and showed it to be a tetraiodo derivative of a compound of phenol and tyrosine or tetraiodothyronine The formulas of thyroxine and of the thyronine nucleus are as follows



Thyronine [4 (4' hydroxyphenoxy) phenylalanine]



Thyroxine [3 5 3' 5' tetraiodothyronine]

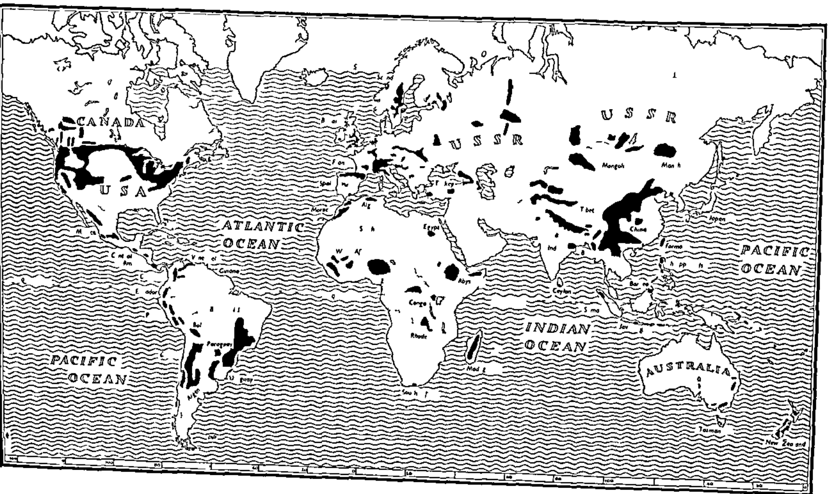
Following the isolation and identification of thyroxine a great number of physiological and therapeutic studies with this and related compounds was carried out which served to establish thyroxine as "the" thyroid hormone Such a conclusion seemed fully acceptable until the important recent announcement of Gross and Pitt Rivers (39) that

3 5 3 L triiodothyronine occurs in the human thyroid and plasma and that this compound may possess as much as three to five times the hormonal activity of L thyroxine Attention had previously been drawn to this substance by Hird and Trikojus (52) when studying chromatograms of an hydrolysate of iodocasein The problem of the relationship between these two compounds and the nature of the thyroid hormone is not yet fully resolved This question is considered later in the section on the circulating thyroid hormone

The first quarter of this century was also extremely productive in two further aspects viz (1) the development of effective means of control of endemic goiter and (2) the demonstration that in an overwhelming proportion of cases this disease is primarily associated, both in man and in farm stock with a deficiency of iodine in the food and water supply in consequence of subnormal concentrations in the rocks and soils of goitrous regions The original large scale experiment for the prevention of goiter in man was carried out in the schools of Ohio over the four years 1916–1920 although as early as 1915 Marine an outstanding worker in this field was teaching that endemic goiter is the easiest known disease to prevent This statement was based upon ten years of experimental study of the chemistry and physiology of the thyroid gland The treatment given in the Ohio experiment—3 grains of sodium iodide taken daily in the drinking water for a period of 10 days in the spring and in the autumn—conclusively demonstrated the truth of Marine s statement Similar prophylactic measures were quickly initiated elsewhere in goitrous regions in the United States Switzerland and other countries with great success and within a few years iodized salt became a recognized form of control In the early years there was some resistance to this form of treatment because of the fear of toxic goiter but careful investigation served to dispel these fears

Surveys carried out in recent years have shown that endemic goiter is very much more extensive than was formerly realized (107) (see map on p 268) * Systematic surveys of sections of the population particularly of the newborn of school children and of service recruits have revealed more and more goitrous areas Where iodine prophylaxis has been introduced and efficiently carried out the disease has been practically abolished but this effect has been shown to become evident only after a number of years of systematic treatment of the population In some goitrous areas the only evidence is a mild or moderate enlargement of

* Map kindly supplied by Iodine Educational Bureau Chilean Nitrate Co London



Goiter Areas of the World

the thyroid which imposes a social handicap but presents no public health problem In other areas the disease constitutes a major social economic and health problem associated with cretinism feeble minded ness, deaf mutism and general physical and mental degeneration In both types of conditions lack of sufficient iodine for the thyroid gland to maintain normal structure and function usually results from an en vironmental deficiency of iodine of differing severity which can be prevented by iodine treatment Physiological and nutritional studies carried out during the last twenty years however have shown clearly that these conditions can also be caused or accentuated by dietary and other factors that interfere with the availability or utilization of iodine or that impose an abnormal demand on the thyroid gland The nature and significance of some of these factors are considered in subsequent sections

This latter period witnessed a number of notable advances in our understanding of the physiology of iodine and thyroid function many of which have not yet been widely applied to the problem of endemic goiter A variety of potent antithyroid agents was discovered in 1943 (7) These have not only proved valuable in the treatment of hyper thyroidism but have been extremely useful tools in the study of thyroid function Studies of iodine metabolism were very greatly facilitated also by the discovery of radioactive isotopes of iodine which permit the use of physiological quantities of this element under physiological con ditions which tag" a given dose with respect to time and which enable accurate measurement of iodine in the body tissues and fluids of intact animals With no other trace element has the advent of radioactive isotopes proved such a powerful weapon in unravelling metabolic secrets At first only I^{128} which has the very limited half life of 26 minutes was available to researchers but later I^{131} with the very satisfactory half life of 8 days was produced in quantity This isotope has been in corporated into both inorganic iodides and calorigenically active organic substances for metabolic investigations

Mention should also be made of the recent development from the method of Sandell and Kolthoff in 1937 (99a) of exceedingly sensitive catalytic colorimetric methods of estimation of the minute concentra tions of iodine present in biological tissues and fluids The application of such methods together with the tracer isotope studies mentioned in the previous paragraph have been potent factors in facilitating our understanding of the relation of thyroid activity to iodine movements in the body Concurrent application of chromatographic methods to the separation of organic constituents of the thyroid and plasma has proved

equally valuable It was by such means that the significance of triiodo thyronine was revealed

Side by side with these investigations, which were stimulated primarily by the need to understand the nutritional physiology of iodine in humans, collateral studies on iodine in relation to the health and productivity of farm stock were proceeding The relation of individual, breed seasonal, and geographical variations in the level of thyroid activity to such productive functions as milk yield egg yield and rates of live weight gain in animals have been studied as well as the control of these functions by the use of artificially iodinated proteins Very limited consideration of this work or of the pharmacological aspects of iodine is possible in this context

II Iodine in Animal Tissues and Fluids

1 *Distribution of Iodine Throughout the Body*

All body tissues and secretions that have been examined have been shown to contain iodine and there is little doubt that it occurs in every cell of the body The total amount present in the healthy human adult has been calculated to lie within the normal range of 20–50 mg (108) Of this small total a high proportion variously estimated at 20–40% is concentrated in the thyroid gland The extent of this concentration which is unique for any trace element in any tissue can be gaged from the fact that the mass of this gland is normally only about one five hundredth or 0 2% of the whole body The skeletal muscles rival the thyroid in total amount of iodine present This is entirely due to their large mass because the iodine concentration of the muscles is usually less than one thousandth of that of the thyroid The pituitary gland which is so intimately related to thyroid function has a very low iodine content This is true also of the central nervous system (68) The concentration in the ovaries however is apparently three to four times that of the muscles and is appreciably higher than in any other extrathyroid tissue with the possible exceptions of the bile and the hair A distribution of this type applies not only to the human but to all species of animals studied (68 72 108) The form or forms in which iodine occurs in the ovaries are unknown Nor is it yet known if this relatively high concentration of iodine serves any particular function in this site There is some evidence of a cyclic change in ovarian iodine in accordance with ovarian activity and of lower values before puberty and after the menopause in women but these observations together with many of the determinations of iodine distribution throughout the tissues need to be repeated with improved modern methods of analysis

The concentrations referred to in the previous paragraph are for total iodine, i.e. inorganic iodide and organically bound iodine. Inorganic or iodide iodine is normally present in the tissues in extremely low concentrations of the order of 1–2 μg % or 0.01–0.02 p.p.m. (96). This fraction of the tissue iodine is in equilibrium with the circulating iodide of the body fluids. It appears that iodide ions, like chloride ions, permeate practically all tissues and are distributed in extra cellular fluids in a manner similar to that described for chlorides by Peters (84). Therapeutic administration of iodide, both at the prophylactic levels necessary for the treatment of simple goiter and at the much higher levels used for exophthalmic goiter, result in greatly increased concentrations of inorganic iodide in tissues and fluids (96). This increase may be sufficient in the blood to invalidate serum protein bound iodine determinations made for diagnostic purposes, as will be shown when considering iodine in the blood. It is likely also that the liberal use of iodized mineral mixtures or "licks" for stock may similarly invalidate blood iodine estimations in these species.

The levels of organically bound iodine in the tissues depend upon the tissue concerned. The forms and amounts of such organic iodine in the thyroid and in the blood are discussed below. In most tissues the concentrations are like those of inorganic iodide, extremely small. A level of about 5 μg % appears to be normal for skeletal muscle (96) but a great deal remains to be learned about the nature and function of this fraction. Its solubility is different from that of thyroxine added to tissue extracts and its distribution is not uniform between the muscle protein fractions of Szent Gyorgyi (myosin and actin) but, most significantly, the amounts decrease in hypothyroidism and increase in hyperthyroidism (97). It seems probable that much of the organic iodine of the tissues consists of thyroxine loosely bound to protein. The application to the tissues, such as the muscles, of the new techniques of paper chromatography and autoradiography, which have been principally responsible for the detection of new iodinated compounds, including triiodothyrone, in the thyroid and plasma, should assist greatly in determining the nature of the compounds present.

2. *Iodine in the Thyroid Gland*

The concentration of total iodine in the thyroid varies widely with the iodine intake, with the activity of the gland, with age and with the individual, but there is no conclusive evidence that it varies significantly with sex in any species. The iodine absorbed from the daily diet overshadows all other factors in determining the thyroid iodine

equally valuable It was by such means that the significance of triiodothyronine was revealed

Side by side with these investigations which were stimulated primarily by the need to understand the nutritional physiology of iodine in humans, collateral studies on iodine in relation to the health and productivity of farm stock were proceeding The relation of individual breed, seasonal, and geographical variations in the level of thyroid activity to such productive functions as milk yield, egg yield and rates of live weight gain in animals have been studied as well as the control of these functions by the use of artificially iodinated proteins Very limited consideration of this work, or of the pharmacological aspects of iodine is possible in this context

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2 *Iodine in the Thyroid Gland*

The concentration of total iodine in the thyroid varies widely with the iodine intake with the activity of the gland with age and with the individual but there is no conclusive evidence that it varies significantly with sex in any species The iodine absorbed from the daily diet overshadows all other factors in determining the thyroid iodine

content. Variation among species is small, except that sea fish have thyroids very much richer in iodine and rats have thyroids slightly poorer in iodine than most mammalian species. The normal healthy thyroid of mammals contains 0.2%–0.5% I on the dry basis, giving a total of about 8–10 mg iodine for the adult human thyroid. This amount may be reduced to as low as 1 mg or less in endemic goiter, but the concentration is even more significantly reduced than the total content because of the compensatory hyperplastic changes which take place in the gland in this condition (Table 36).

The concentration of iodine in the thyroid has long been used as a criterion of its capacity to function effectively. Many years ago it was shown by Marine and Lenhart (71) that when the concentration falls below 0.1% hyperplastic changes are regularly found. This figure may therefore be regarded as close to the minimum effective level in the gland. Marine's claim has been substantially confirmed in more recent work with sheep and pigs (3). A significant inverse correlation was found between the iodine concentration and the height of the thyroid epithelium in these species. Sheep's thyroids with marked hyperplasia contained 0.01% I on the dry basis, those with moderate hyperplasia 0.04% I, and pig's thyroids showing only very slight hyperplasia 0.11% I on the dry basis. Feeding stabilized iodized salt to these animals completely eliminated the hyperplasia of the follicular epithelium and increased the iodine content of the glands so that few contained less than 0.2% and none less than 0.12% (3).

Iodine exists in the thyroid in a number of forms, not all of which have yet been identified. Those which have been detected are inorganic iodide, thyroxine, 3,5,3′ triiodothyronine, monoiodotyrosine, diiodotyrosine, polypeptides containing thyroxine, and thyroglobulin. Inorganic iodide constitutes only about one tenth of the total iodine present in the normal gland, whereas thyroglobulin, the thyroid colloid, which is the storage form of the thyroid hormone, contains a very high proportion of the total iodine of the normal gland (96). Thyroglobulin is a very large molecule, comparable in size to gamma globulin of blood plasma, but it is not yet clear whether it is a chemical entity in the sense that the crystalline blood proteins are entities. Thyroxine and diiodotyrosine do not appear to exist in the free state in significant concentrations but are bound in peptide linkage to the polypeptide chain of the protein. The amounts and proportions of these amino acids, and therefore the iodine content of thyroglobulin, vary with the supply of iodine to the gland. The native protein of the thyroid gland must thus be considered primarily as a storage form of iodine.

TABLE 36

WEIGHT AND IODINE CONTENT OF NORMAL AND GOITROUS THYROID GLANDS

Description	Number of samples	Weight of gland (g)		Iodine in gland (%)		Total iodine in gland (mg)
		Fresh	Dry	Fresh	Dry	
Human						
Normal glands	20	21.9	5.05	0.017	0.203	10.2
Nontoxic nodular goiters	15	99.7	20.00	0.021	0.105	21.0
Normal glands[b]	124	20.7	4.46	0.045	0.204	9.1
Hyperplastic goiters	93	55.8	9.76	0.017	0.091	9.2
Sheep[b]						
Normal glands	19	7.0	1.95	0.069	0.247	4.8
Moderate hyperplasia	1	48.0	10.92	0.009	0.040	4.4
Marked hyperplasia	6	77.6	12.83	0.0001	0.0006	0.08
Cattle[b]						
Normal glands	17	11.9	3.84	0.112	0.348	13.3
Early hyperplasia	5	19.2	4.34	0.023	0.100	4.3
Marked hyperplasia	5	103.2	22.38	0.004	0.019	4.2
Dog[b]						
Normal glands	3	2.08	0.52	0.078	0.332	1.4
Moderate hyperplasia	9	7.36	1.70	0.008	0.037	0.5
Marked hyperplasia	18	16.45	3.59	0.002	0.011	0.3

[a] Gutman *et al* (41) [b] Marine and Lenhart (71) [c] Oswald (82)

The thyroid gland serves four main functions with respect to iodine Firstly, it traps with very great efficiency the inorganic iodide of the body obtained from the food and from the reversion of tissue and circulating hormonal iodine during the course of metabolism Secondly, it oxidizes this iodide to iodine, incorporates the iodine into tyrosine to form diiodotyrosine and couples two molecules of diiodotyrosine to give thyroxine These two processes are under the control of thyrotropin Thirdly, it serves as a reservoir for thyroid hormone, which it fixes and stores as thyroglobulin, and regulates the release of this hormone into the circulation, also under the control of thyrotropin (40, 44 96) Fourthly, on the basis of recent evidence, it forms 3,5 3 triiodothyronine and releases this hormonally active compound into the circulation (40) Stated in the simplest terms, the functions of the thyroid gland are the formation the storage, and the release of the iodine containing hormone or hormones *These can only proceed without compromise in function* or adjustment in thyroid morphology when the gland is supplied with sufficient iodine

Certain factors influencing the accumulation of iodide by the thyroid and its conversion into organically bound iodine are discussed later in the section dealing with iodine metabolism but a detailed consideration of the enzymatic and chemical mechanisms involved in the four functions of the gland which have just been mentioned lies outside the scope of this book An appraisal of recent work on the biochemistry of the thyroid gland, in which these functions are critically discussed has recently appeared by Gross and Pitt Rivers (40)

3 *Blood Iodine*

Extremely variable values ranging from 3 to 30 μg per 100 ml, have been reported for the total iodine content of the whole blood of normal humans although a high proportion of these values lies between 8 and 12 μg per 100 ml (25) Very similar average concentrations of total iodine occur in the whole blood of most animal species, but the individual variability is so great that significant species differences if they exist are difficult to determine The consumption of diets especially rich in iodine due to the inclusion of large amounts of sea foods or seaweeds results in total blood iodine levels higher than normal A similar rise occurs when iodides are being administered Therapeutic doses of iodide (e g 1 g of NaI) may raise the level temporarily by several hundred per cent (96)

a *Clinical Diagnosis* Attempts to use total blood iodine determinations in the clinical diagnosis of thyroid disease and in studying thyroid

function have been disappointing. This was due until recently to the tedious and unsatisfactory nature of the methods available and to a failure to realize that total blood iodine is composed of at least two distinct fractions, inorganic iodide and protein bound iodine, which do not vary in the same way with varying thyroid activity. Ionized (inorganic) iodine is normally extremely low in the blood (1–2 μg per 100 ml), as in all extrathyroidal tissues. Moreover, this fraction remains approximately constant in different states of thyroid activity (96). It can, however, increase markedly when exogenous iodine enters the organism, as in the treatment of thyroid dysfunction with iodide. Salter and his collaborators (98) have drawn attention to the "false" increase in protein bound iodine, leading to diagnostic confusion, which can occur under such conditions unless appropriate precautions are taken in the analytical procedures employed.

The protein bound iodine fraction of the blood, in contrast to the inorganic iodine fraction, is very sensitive to changes in the level of thyroid activity. Exceedingly delicate and relatively rapid catalytic *methods of determining protein bound iodine in as little as 0.5–3.0 ml* of serum or plasma are now available (23, 99). The development of these methods, which make use of the power of iodide to catalyze the reduction of ceric to cerous ions by arsenious acid, represents, as was mentioned earlier, an advance of very great significance to an improved understanding of the relation of blood iodine levels to thyroid activity. Serum or plasma is almost invariably used for these determinations rather than whole blood, although reports on the distribution of iodine between the red cells and plasma are conflicting (89).

b. *Protein Bound Iodine.* The protein bound (PBI) or the serum precipitable iodine (SPI) of normal human blood serum or plasma is much less variable when estimated with modern methods than the total blood iodine as found by earlier workers with the less satisfactory methods then available. The limits of normality may be stated as 4–8 μg per 100 ml, with a mean value lying between 5 and 6 μg per 100 ml (42, 89) (Fig. 22). Slightly lower norms (3–4 μg per 100 ml) exist in the mouse, rat, dog, and domestic fowl (109) and also in adult sheep (112) and dairy cattle (66, 69). In beef cattle the average PBI appears to be still lower (2–3 μg per 100 ml), judging by the results of one investigation (69), but a later study disclosed mean values for Hereford and Angus bulls very close to 4 μg per 100 ml (59). Significant differences between the sexes or among the dairy breeds have not consistently been obtained, but there is a highly significant decrease with age in dairy cattle.

Lewis and Ralston (66) suggest from the results of their investigations that the normal range of PBI concentrations of dairy animals of various ages be tentatively set as follows: calves under 2 days, 8.0–18.0 μg per 100 ml; animals between 2 days and 12 months, 3.5–12.0 μg per 100 ml; 13–24 months of age, 3.5–10.0 μg per 100 ml; and cows over 24 months,

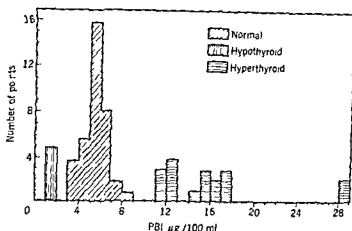


FIG. 22. Distribution of protein bound iodine (PBI) in normal subjects and in patients whose thyroid disease was confirmed by a satisfactory response to appropriate treatment. (Hallman *et al.* 42.)

3.0–8.0 μg per 100 ml. In a later more detailed study these workers (67) found the PBI values of young calves to be much higher during the first 48 hours post partum than at any later time. This was shown to be due to the ingestion of colostrum which contains relatively large amounts of protein bound iodine during the first 12 hours after calving (67). The mean PBI of the serum of 7 calves within the first 12 hours of birth *before* ingesting any colostrum was found to be 8.9 μg per 100 ml, compared with 15.0 μg per 100 ml for the same 7 calves *after* nursing. This represents a statistically significant increase of 66%. The magnitude of the age changes in the plasma PBI of calves up to 18 months of age are given in Table 37.

In man PBI levels do not appear to be significantly influenced by sex or by age, although a critical series of values for infants and very young children such as was quoted for calves in the preceding paragraph does not seem to have been carried out. Menstrual changes are small and inconsistent but there is a significant rise in PBI during human pregnancy. Heinemann (47), Hallman (42), and their collaborators have shown that as early as the third week in human pregnancy PBI values rise sharply to levels in the upper range of normal or even to levels which outside of pregnancy are characteristic of hyperthyroidism. They

have shown further that if such a rise does not occur the pregnancy is likely to end in abortion. This rise in PBI levels is not attended by any clinical evidence of excessive activity of the thyroid and occurs well before the increase in BMR of pregnancy. This interesting phenomenon in pregnant women and the position during pregnancy in other

TABLE 37
CHANGES IN THE CONCENTRATION OF PLASMA PROTEIN BOUND IODINE WITH ADVANCING AGE OF CALVES (67)

Age	Number of calves	Number of determinations	Average µg %	S
Under 24 hours	22	22	14.8	5.3
24–48 hours	8	8	10.8	2.3
3– 4 days	8	9	7.9	4.2
5– 7 days	9	9	7.6	4.1
8–30 days	24	34	6.9	3.6
1– 3 months	18	18	6.2	2.6
4– 6 months	11	17	8.1	3.4
7–12 months	21	27	7.3	1.6
13–18 months	10	27	6.8	2.7

species deserve further study. Limited evidence suggests that no such rise in PBI occurs in pregnant cows (65).

The relationship which exists between the level of thyroid activity and the level of PBI in the serum of humans is, however, of the greatest interest and importance. The highly significant increase in levels above normal in hyperthyroid states and equally significant depression below normal in hypothyroid states have been demonstrated by many workers (89, 96) (Fig. 22). The rise in hyperthyroidism and the fall in hypothyroidism illustrated in Fig. 22 occur even when these conditions are not associated with corresponding changes in the BMR. Such changes in PBI do not normally occur when hypometabolism and hypermetabolism are not of thyroid origin. It is therefore obvious that PBI determinations carried out with proper precautions are an extremely valuable aid in the clinical diagnosis of thyroid dysfunction. Rapport and Curtis (89) are careful to point out, however, that "diagnosis of thyroid dysfunction cannot be made from it [PBI] alone any more than a diagnosis of acute appendicitis can be made from the total and differential leucocyte count."

Attempts have been made to use PBI determinations in immature beef cattle to predict future live weight gains (59). Hypothyroidism has been associated with dwarfism and hence low rate of gain (16) and it would

expected that there would be an optimum PBI level commensurate with satisfactory growth and efficiency of feed use if this level is an index of thyroid activity. A highly significant correlation between rate of metabolism in male beef calves and their plasma PBI levels has been observed and also between the efficiency of gain in 10 Hereford bulls and their PBI levels (59), but very much further study is necessary before the value of PBI determinations in the prediction of future gains or of other production potentials in farm animals can be assessed.

The Circulating Thyroid Hormone

For years the nature of the circulating thyroid hormone has been the subject of speculation. The organic iodine of the plasma is nondialyzable and is loosely bound to one of the plasma proteins. Early evidence that albumin carried the iodinated compounds has been disproved and it now seems that small amounts of α globulin contaminating the albumin actually constitute the binding protein. This was demonstrated by the use of a combination of paper electrophoresis and autoradiography with normal human plasma, with and without added synthetic radiothyroxine of high activity (33). Both the natural and the radiothyroxine were found to be associated with a protein having a mobility similar to that of α globulin. Of great interest is the report that triiodothyronine does not appear to be specifically associated with this protein in the circulation (40).

Following the investigations of Taurog and Chaikoff (110), Laidlaw (...), and others it became generally accepted that thyroxine (bound to protein) was the circulating hormone and that this compound constitutes the PBI of the serum, or at least the effective part of it. This conclusion requires revision in the light of the demonstration of the high thyroidal activity of triiodothyronine and of its presence in addition to thyroxine in the plasma (39). Whether thyroxine is the precursor of triiodothyronine in the thyroid and is itself physiologically inactive, or whether both compounds possess activity independently of their relation to each other, remains to be determined. For the present, as Gross and Pitt Rivers (40) have stated, the circulating thyroid hormone must be regarded as being composed of both thyroxine and triiodothyronine.

In this connection it should be mentioned that thyroid hormone is a generic term indicating any substance that will relieve human myxedema and whose administration causes a specific series of biological events. A number of such substances are known of which triiodothyronine and thyroxine are the most potent examples. These substances vary in their physical and chemical constitution but all contain a single common

denominator which is essential for physiological activity This is the chemical group known as thyronine (see p 266) All thyroactive substances contain the thyronine nucleus and all those prepared from natural sources or with a substantial degree of activity contain iodine substituted in the inner aromatic ring Removal of *both* the outer iodine atoms (3 5) from thyroxine results in a loss of 90% of the activity (2) These facts indicate the essential nature of both iodine and thyronine in the thyroid hormone although both tetrabromothyronine and tetrachlorothyronine exhibit weak thyroidal activity

5 *Iodine in Milk*

The total iodine content of milk is highly variable in all species studied Values have been reported for cow s milk ranging from 0 to 100 μg per 100 ml Some of the reported variability is undoubtedly associated with analytical errors and with contamination but the level of dietary intake of iodine is the main determining factor In fact the iodine concentration of the milk produced in a given locality has been shown to be quite a good index of endemic goiter incidence in that region (79) Iodide supplements are capable of raising the level of iodine in milk far above normal Such treatment of dairy cows has been suggested many times as an effective means of raising the iodine intake of children and of pregnant women in goitrous areas

The great variability in iodine concentration in milk makes it difficult to determine "normal levels A range of 3–8 μg per 100 ml however includes a high proportion of the values obtained for normal cow s milk in various investigations Thus Lewis and Ralston (67) in the United States reported a range of 6 7–13 6 μg per 100 ml for the total iodine of nine healthy cows receiving normal rations unsupplemented with iodized salt or mineral mixtures Orr and Leitch (78) in England raised the iodine content of milk from 4–7 μg per 100 ml to 33 μg per 100 ml by adding 0 18 g iodine as KI daily to the diet of the cows and Blom (12) in South Africa found that a supplement of 0 1 g KI daily raised the iodine content of cow s milk from a normal 2–7 μg per 100 ml to 51–107 μg per 100 ml Therapeutic doses of iodide of 1 g or more given to lactating women can result in milk containing several milligrams of iodine per 100 ml (96)

The iodine content of goat s ewe s and human milk appears to be very similar to or perhaps slightly higher than that of cow s milk but the values reported are so variable that valid species comparisons appear impossible Salter (96) quotes values of 5–24 μg per 100 ml for the iodine content of human colostrum and 4–8 μg per 100 ml for human

milk once lactation is established The position of colostrum and true milk in respect to iodine concentration is curious in that there is appreciable evidence for several species that milk is richer in this element than colostrum This is not supported by a recent study (67) in which the total iodine of the colostrum of five cows ranged from 20 μg to over 35 μg per 100 ml, whereas the later milk of the same five cows ranged in iodine content from 7 2 to 13 6 μg per 100 ml This question merits further investigation with modern methods of analysis especially in view of the possible importance to the newborn of the colostral iodine suggested by the work of Lewis and Ralston (67) with cows

Much remains to be learned of the forms in which iodine exists in milk It has been shown that milk iodine occurs almost entirely in the skim milk fraction and not in the fat and that it is precipitated with the milk proteins No thyroxine like fraction can be isolated from milk and the normal bovine mammary gland has been shown to be impervious to thyroxine (90) Normal cow s milk on the other hand contains one half or more of its total iodine in organic combination as determined by the standard procedures used for estimating serum PBI and cow s colostrum contains an even higher proportion of its total iodine in this form (67) * The nature of the organic iodine of milk and colostrum presents an intriguing problem, especially as neither milk nor colostrum possesses calorigenic properties

6 *Iodine in Eggs*

Considerable attention has been devoted to the iodine in hen s eggs mainly because it supplies the whole of the requirements of this element for the developing chick embryo but also because of an interest in eggs as a source of iodine in human dietaries On average diets, hen s eggs usually contain a total of 0 004–0 01 mg iodine most of which is located in the yolk (93) The amount however is highly dependent upon the intake of iodine by the hen In goitrous areas iodine may be present in much smaller quantities than those just quoted (50) but by feeding the hen sufficiently large amounts of iodine it is possible to increase the iodine concentration in the egg as much as one hundred times the normal levels (113) Eggs containing a total of 1–3 mg of iodine have

* By the use of a chromatographic-radioautographic technique capable of separating monoiodotyrosine diiodotyrosine thyroxine and sodium iodide from skim milk Wright *et al* (116a) have been unable to detect the presence of any iodine compounds other than iodide in the milk of goats treated with I^{131} labeled iodocasein or sodium iodide

been produced by these means and iodized eggs have been used in certain areas for therapeutic purposes

III Physiological Functions of Iodine and the Thyroid Hormone

Since iodine is an essential component of the thyroid hormone and no other function for this element is known it follows that the physiological functions of iodine are identical with those of the thyroid hormone This has been established as a result of studies correlating the effects on animals of naturally occurring and artificial iodine deficient diets and of those of thyroid removal or hypofunctioning of the gland not conditioned by lack of iodine Studies of hyperfunctioning states have also contributed to an understanding of the relationship that exists between iodine and the functions of the thyroid hormone At the same time it is necessary to emphasize that many disorders of the thyroid gland involving a deficient or an excessive production of thyroid hormone are not conditioned by variations in supplies of dietary iodine In these cases iodine metabolism is clearly involved but it is secondary to the glandular dysfunction Such dysfunctions lie largely outside the scope of a text on nutrition and are only considered insofar as they throw light on the nutritional physiology of iodine

The studies just mentioned have revealed that the thyroid hormone and therefore iodine is concerned with a variety of metabolic processes It is apparent that this hormone (a) exercises control over the rate of energy metabolism or level of oxidation of all cells (its calorigenic effect) (b) influences physical and mental growth and differentiation or maturation of tissues (c) affects other endocrine glands especially the hypophysis and the gonads (d) influences neuromuscular functioning (e) affects circulatory dynamics (f) has an effect on the integument and its outgrowths hair fur and feathers and (g) influences the metabolism of the food nutrients including various minerals and water

Many of these functions are related and interdependent Indeed the great variety of thyroid effects is generally interpreted as indicating that the thyroid hormone has a single primary function that of controlling the rate of cellular oxidation and that all other effects are secondary manifestations *of a disturbance in this function affecting various* specialized processes Most of the cardiovascular abnormalities of hypo or hyperthyroidism can no doubt be related in this way to lowered or heightened metabolic rate but some of the thyroid effects are not so easily related to the calorigenic effect This question is considered further in the section dealing with the mechanism of action of the thyroid hormone

1 *Metabolic Processes*

As indicated above the primary and fundamental function of iodine, through its presence in the thyroid hormone, is the control of the rate of cellular oxidation This applies in all vertebrate organisms The rate of energy exchange is conditioned by the thyroid hormone and the quantity of heat liberated by an organism at relative rest is decreased by deficiencies and elevated by excesses of this hormone After total thyroidectomy the basal metabolic rate gradually falls to about one half of its normal value but can be raised by the administration of thyro active substances in proportion to the amount and the potency of the materials which are used Similarly in endemic goiter or in experimental iodine deficiency where the production of thyroid hormone is limited by lack of iodine, the basal metabolic rate of animals is lowered In these latter conditions the administration of either thyroid substance or iodine restores the normal basal metabolic rate

The calorigenic action of thyroid hormone is evidenced by its effect on the oxygen consumption of excised tissues and organs Liver, kidney muscles and other tissues from iodine deficient or thyroidless animals consume less oxygen than normal whereas comparable tissues from hyperthyroid animals show an oxygen consumption greater than normal (15) Tissues excised and cultured *in vitro* are not affected by thyroxine which suggests that tissues maintained apart from the organism are deprived of some organismic factor which is essential for the action of thyroid hormone

Disturbances in the metabolism of water, salt proteins carbohydrates and lipoids have been observed in animals with hypofunctioning and hyperfunctioning thyroids In the former condition there is an increased retention of salt and of water mostly extracellular in location and a considerable reduction in plasma volume A pronounced water and salt diuresis accompanied by an increased plasma volume follows the administration of thyroid hormone Increased nitrogen excretion also takes place largely as a result of the greater breakdown of cells

The role of the thyroid in carbohydrate metabolism is far from clear but its effect on lipoid metabolism is indicated by the relation between the lipoid concentration in the blood and the functional state of this gland Hyperthyroid states are accompanied by low blood cholesterol levels and hypothyroid states by above normal levels of this constituent in the blood Clinicians have recognized for many years that the meta bolic rate is in general, inversely proportional to the blood cholesterol concentration

2 *Growth and Differentiation*

Depriving a young animal of thyroid hormone by surgery or ablation or by feeding diets low in iodine or high in goitrogenic agents greatly retards general growth as a result at least in part of delayed osseous development The dwarfism produced in rats or birds by total thyroid ectomy at an early age is very marked although not so severe as that produced by hypophysectomy In human infants athyreosis leads to the type of dwarfism known as cretinism which at one time was common in goitrous regions Even in cretins administration of dried thyroid can result in increased growth and similar results have been obtained in iodine deficient areas with children who have failed to grow normally because of lesser degrees of hypothyroidism Definite retardation of growth in young people goes hand in hand with goiter in severely goitrous areas In such areas iodine administration during adolescence increases the rate of growth as well as reducing the size and incidence of goiter Many Swiss records could be cited to support this statement but perhaps the most striking are those taken from the Bern Secondary School and submitted to statistical analysis by Stocks (105) Measurements of height weight and thyroid size of 1130 growing girls from this school were obtained for a year before and for 3½ years after treatment with a regular weekly dose of 2 mg sodium iodide in tablet form Large and remarkable increases in mean growth rates amounting to no less than 3 cm and 3½ kg in excess of the mean growth curves were found over this period for girls with pronounced goiters and similarly highly significant increases although progressively less in extent from such treatment of girls with moderate to small goiters The actual increases in mean height and weight of all treated girls *above* the values for girls whose treatment had not yet begun were as follows

	Increase in height (cm)	Increase in weight (kg)
In 1 year	1 000 ± 0 148	0 926 ± 0 144
In 2 years	1 602 ± 0 212	1 578 ± 0 206
In 3 or more years	1 629 ± 0 284	2 021 ± 0 276

Retardation in general growth as a result of thyroidectomy or severe deprivation of iodine is accompanied by a delay in almost all developmental processes This is seen in its most striking form in the effect of thyroid substance on the metamorphosis of amphibian larvae which is of particular interest because it provides evidence of a distinction between the growth and the differentiation effects of the thyroid

hormone It seems that the hastening of metamorphosis of frog tadpoles brought about by thyroid substances is not due to a mere stimulation of metabolic rate For instance the dinitrophenols elevate metabolism in tadpoles but they do not hasten their development into frogs The relation of the thyroid hormone to maturation or development is apparent however in all species A hypofunctioning thyroid retards mental development in the human species and results in depressed reproductive activity or failure in the development of the male and female gonads and the secondary sex organs of the young of all birds and mammals These latter effects of thyroid insufficiency are considered in the following sections

3 *The Neuromuscular System*

Human hypothyroidism is characterized by retarded mental development in the young and mental dullness and apathy in both young and old In hyperthyroidism, by contrast there is emotional instability, nervousness, and irritability, accompanied usually by muscle tremors hyperactivity of the sweat glands, and increased peristaltic activity of the intestines These effects are taken as evidence that the thyroid hormone affects both the central nervous system and the autonomic system Numerous experiments on animals have, in fact, demonstrated that the thyroid secretion sensitizes the nervous system Moreover, it is well known that hypothyroids have a lowered resistance to narcotics and increased thresholds to light and sound stimuli Direct experimental support for such an effect has been provided by electroencephalographic studies demonstrating that in myxedema and other thyroid deficiencies the cerebral cortex originates fewer alpha waves per second than the normal brain The electrical activity of the brain can be returned to normal by the administration of thyroxine, or can even be increased beyond normal if sufficient thyroid hormone is given Ross and Schwab (94) found a good correlation between the BMR and the rate of emanation of alpha waves from the nervous system

One of the simplest and most obvious examples of the influence of the thyroid hormone on nervous tissue functioning is that affecting the respiratory centers Following thyroidectomy, or in the hypothyroid state whatever its cause the respiratory centers in the medulla are less sensitive to carbon dioxide and the rate of breathing is diminished Treatment with thyroid substance or thyroxine results in a rapid restoration of the sensitivity of these centers with an increased respiratory rate The mechanism involved in this sensitizing of the nervous system by the thyroid secretion is unknown but it seems probable that it is not un

connected with changes in the rate of oxidation of this tissue, such as have already been demonstrated for the brain in different thyroid states (20)

From the earliest times statements have been made associating stupidity mental dullness or lack of intelligence with the incidence of goiter Controlled studies using modern intelligence tests or similar ratings have however given very variable results when these have been applied to school children with normal slightly enlarged or markedly enlarged thyroids Thus Shee (100a) working in an area of high endemic goiter in Ireland found a significant inverse correlation between intelligence and established goiter whereas Stocks *et al* (106) could not find any evidence that proficiency in school work is lowered in adolescents with enlarged thyroids

4 *Relation of the Thyroid to other Endocrine Glands*

Complex interrelationships are characteristic of the activities of all the endocrine glands With the thyroid such interrelationships are conspicuous and far reaching in their physiological effects The normal production storage and release of the thyroid hormone in which a series of complicated biochemical processes involving iodine is involved are believed to be governed by a delicate balance and interaction between the thyroid hormone and thyrotropin of the anterior lobe of the hypophysis Increasing amounts of thyroid hormone result in atrophic changes in the anterior hypophysis and diminish the secretion of thyrotropin Thyroidectomy or hypothyroidism resulting from inadequate iodine or from treatment with goitrogenic substances and the consequent diminished thyroid output stimulates the anterior pituitary to produce and release increased amounts of thyrotropin When bodily needs for thyroid hormone cannot be met as in iodine deficiency this *increased amount of thyrotropin stimulates compensatory enlargement* of the thyroid gland Such an enlargement, or goiter is an attempt to increase the secretory surface of the thyroid follicles evidenced histologically by hypertrophy and hyperplasia

The thyroid pituitary interrelationship is shown further by the effects of hypophysectomy and of parenteral administration of thyrotropin Hypophysectomy results in atrophy of the thyroid gland because of the withdrawal of thyrotropin from the organism This can be prevented by the administration of suitable pituitary extracts whereas the administration of such extracts to the normal animal produces gross enlargement of the thyroid and increases the flow of blood to this gland It has been shown moreover that thyrotropin produces hypertrophy of

thyroid cells when these are grown *in vitro* in blood serum (1) The interesting question of direct thyrotropin effects on metabolism independent of the thyroid lies outside the scope of this book

Marked cytological changes in adrenal cortical cells have been observed in both hypo and hyperthyroidism (26) Thyroidectomy in the rat is followed by a reduction in the size of the adrenals, whilst administration of thyroid hormone by mouth or parenterally will result in adrenal cortical hypertrophy (53) Conversely, hyperfunction of the adrenal cortex may result in a decrease in thyroid function, presumably due to a decrease in thyrotropin, and the administration of epinephrine may induce hyperplastic changes in the thyroid (101) In the adrenalectomized animal the uptake of I^{131} is increased following the administration of adrenalin, whereas in the intact rat such treatment is followed by a decreased uptake

An interrelationship between the thyroid and the sex glands is established by a wide range of data In the human organism, colloid goiter not infrequently develops during puberty and hyperthyroidism is not uncommonly precipitated at the menopause Cretins are usually sterile and invariably fail to develop normal sexual vigor, with a delayed maturation of the genitalia In all birds and mammals thyroidectomy at an early age is followed by a long period in which the gonads and the secondary sex organs remain in an infantile condition

Thyroid gonadal interrelationships can be very conspicuous in some types of birds due to plumage changes as well as to effects on other secondary sex characters In the Brown Leghorn male for instance total thyroidectomy is followed by a long period in which the testes remain small and free from spermatozoa The comb decreases in size molting is inhibited and the characteristic male plumage pattern is lost Administration of estrogen to such animals does not induce the female plumage pattern as it does in normal males which suggests that this pattern results from the synergistic action of the thyroid and ovarian hormones (11) These gonadal effects of thyroidectomy in birds can be overcome by treatment with thyroid hormone

Other manifestations of a relation between the level of thyroid activity and the functioning of the gonads or secondary sex glands occur in farm stock and these are of considerable practical importance In goitrous regions reproductive failure revealed particularly in the birth of hairless, weak or dead young is the outstanding and often the only symptom of iodine deficiency and consequent impairment of thyroid function in farm animals A seasonal reduction in egg production in poultry or in sperm production in rams associated with periods of very

high environmental temperatures has also been related to a mild hypothyroid state but *there is no evidence that they are necessarily due to reduced intakes of dietary iodine* Administration of appropriate doses of thyroproteins (but not of iodine) prevents these depressions in reproductive functioning and administration of thiouracil enables them to be simulated (14) It has been shown further, that thyroidectomy reduces milk yield in cows and goats and that treatment of normal animals with thyroxine or thyroproteins under certain conditions can induce significant increases in milk production and changes in milk composition The practical possibilities of thyroproteins in animal production and the question as to whether these effects are merely secondary to the calorigenic action of thyroid cannot be considered here

5 *Effects on the Integument*

Hypofunctioning of the thyroid whatever its cause is frequently associated with changes in the skin hair or feathers some of which have already been referred to Cretins exhibit a pale gray skin lacking in mobility and their hair is usually dry and scanty In myxedema drying roughening and thickening of skin and shedding of the hair are characteristic symptoms Piglets born in goitrous areas are hairless and have thick puffy skins In birds the relation of different levels of thyroid activity to the molting process and to the form structure and pigmentation of the feathers can be particularly striking Thyroid gonadal relations are intimately concerned in these changes as was mentioned in the preceding section Thyroidectomy reduces the growth rate of feathers inhibits molting and results in a loss of the characteristic male and female plumage pattern (11) Excessive thyroid hormone on the other hand reduces the growth rate of the feathers and accelerates molting The plumage responses vary with the species and the breed and feathers on different parts of the body do not respond alike

6 *Mechanism of Action of the Thyroid Hormone*

It is generally accepted that the thyroid hormone controls tissue metabolism through the regulation of various enzyme activities but the mechanism through which this is achieved is still not understood It could act through the indirect release of some unknown metabolically active substance but the more likely alternative is that it causes the release of coenzymes protein carriers or other substances needed for many of the energy transforming processes of the cells Direct involvement of thyroxine in an enzyme system has however never been demonstrated Indeed Barker (9) has stated it seems quite doubtful if

one substance could act specifically in each one of such a diversity of enzymes as the cytochrome system, succinoxidase, apyrase, arginase and xanthine oxidase, all of which have been shown to be affected in various tissues, by variations in thyroid activity or by treatment of the animal with different amounts of thyroactive substances (9) Barker has further pointed out that the concentrations of thyroxine in the tissues, based on calculations of the amounts of DL thyroxine or L thyroxine required to return the depressed BMR to normal, are far smaller than those of such well established cofactors as riboflavin, niacin pyridoxine or pantothenic acid He claims that it is difficult to see how such minute amounts of the hormone could carry out a specific function with each of several enzyme systems" Recent evidence indicating that the more active triiodothyronine may, in fact, be the effective thyroactive substance within the tissues gives added significance to these calculations

IV Iodine Metabolism

The distribution of iodine throughout the tissues, and its relation to the formation release and circulation of the thyroid hormone have been considered above In this section the question of iodine movements within the body—its absorption retention, and excretion in different thyroid states—is discussed Radioiodine, especially I^{131}, has proved an exceptionally useful tool in studies of these aspects of iodine metabolism Such studies have involved the use of inorganic iodides containing radioactive iodine and various calorigenically active substances containing the labeled iodine in organic combination The former can be regarded as taking part in an anabolic phase and the latter in a catabolic phase of iodine metabolism

1 *Absorption of Iodine*

In foods of vegetable origin most of the iodine appears to be present as inorganic iodide, whereas in foods of animal origin the iodine exists partly in inorganic form and partly in organic combination Human beings therefore ingest iodine predominantly as iodide Inorganic iodide administered by mouth is rapidly and more or less completely, absorbed from the small intestine In fact at the end of a given interval after oral ingestion about the same amount of iodine enters the thyroid gland as after subcutaneous injection (62) Absorption takes place at an exponential rate which varies more or less in direct proportion to the level of thyroid activity (55) Ordinarily very little (about 0 2%) of it appears in the feces

Rather less is known about the absorption of organic iodine but there

is evidence that physiologically active compounds are less completely and more slowly absorbed than inorganic forms of this element and that the rate of absorption varies somewhat with the metabolic level of the subject Absorption is faster for example in the normal than in the hypothyroid individual Moreover a substantial proportion of the dose (11–60%) appears in the feces all of which is organically bound (55) From work done with diiodotyrosine and thyroxine it seems that some of the organically bound iodine is absorbed as such and the rest is broken down to iodide in the alimentary tract Most of the iodine normally entering the circulation may therefore be assumed to be in ionic form and the principal factor determining the rate and extent of its passage into the blood is the thyroid state of the individual Little is known of other factors affecting iodine assimilation other than high levels of dietary calcium which possibly depress iodine absorption The question is considered again in Section VII of this chapter

2 *The Fate of Absorbed Iodine*

Absorbed inorganic iodide disappears from the blood at an exponential rate which is the sum of the individual rates of removal by the thyroid by the kidneys and by other tissues Each of these takes place at rates proportional to the concentration of iodine in the blood (55) An idea of the relative importance of these three pathways can be obtained from the fact that in normal human adults the thyroid accumulates iodide at the rate of about 2.5% per hour the kidneys excrete it at the rate of about 6% per hour and the rest of the tissues of the body at about 1–2% per hour (55) The remarkable concentrating power of the thyroid the "iodide trap to which earlier reference has been made varies greatly with the activity of the gland In exophthalmic goiter the rate of accumulation by the thyroid approximates 20% per hour or 8 times the rate of the euthyroid individual Although iodine is accumulated faster and to a larger degree by the thyroid in hyperthyroidism it is soon lost as the hyperactive gland expends its synthesized hormone

The appearance and disappearance of tracer iodide in the thyroid affords a useful means of appraising the activity of the gland and various tests involving the use of I^{131} have been evolved for the diagnosis and treatment of thyroid dysfunction The tests were at first confined to direct measurement of the proportion and the rate of uptake in the thyroid In general it was found that normal people concentrate less than 40% of the dose and thyrotoxic patients concentrate more than 40% However the considerable overlapping which is general in these tests greatly limits their usefulness

The best determination of function in an organ which is removing a substance from the blood is its plasma clearance The thyroidal iodide clearance," defined as the volume of plasma cleared of its iodide per minute, has been extensively studied in man In the early stages of investigation calculation of this clearance required the simultaneous determination of the thyroid and blood iodine but improved techniques have been developed in which measurements are made over the thyroid and over a part of the body which does not concentrate iodine, such as the thigh (77) In normal individuals the thyroid clears an average of 10–20 ml /min, whereas the average exophthalmic goiter clears as much as 130 ml /min (96) The diagnostic sensitivity of this index can be gaged from the fact that the maximal accumulation in Graves disease rarely exceeds three times the euthyroid level On the other hand, the thyroidal clearance test and indeed all tests with radioiodine are much less sensitive to myxedema than to thyrotoxicosis—so much so that many workers have reported difficulty in distinguishing myxedema from the normal (115) For this purpose, as is shown below a method which uses the 24–48 hour iodine excretion appears especially valuable

Iodine enters the thyroid gland as iodide and at normal intakes is rapidly incorporated into organic combinations through the series of transformations mentioned earlier When an excess of iodide is available a large amount may remain in the gland for some time before organic transformation occurs Following the initial period of concentration after administration of a tracer dose, the iodine is slowly released into the circulation and the content in the normal thyroid falls by about 0 6%/day in thyrotoxicosis the rate is about 10 times as great (86) Within a few days the plasma activity rises to a steady value which is maintained for some time Almost all of this activity is due to protein bound iodine presumably as hormone (86)

3 *Excretion of Iodine*

Orally administered iodide as stated above is almost completely absorbed with very little of it appearing in the feces This indicates that the kidney is the main pathway of excretion of iodide Studies with radioiodide have shown that some of the absorbed iodide is excreted into the stomach but since the gastric iodine is not recoverable in the feces it must be reabsorbed farther down the gastrointestinal tract (62) Excretion of iodide into the stomach may therefore be considered as a mechanism for conserving iodine by delaying its passage to the kidneys and consequent excretion in the urine

The excretion of the iodine of organic iodine compounds has been

studied by means of labeled thyroxin both at and above physiological levels At high dose levels the absorbed thyroxin is partially deiodinated in the tissues and the iodine excreted in the urine as iodide Part of the thyroxine is also taken up by the liver and excreted through the bile into the feces unchanged or in organically bound form The proportion of the administered organic iodine appearing in the feces may be very high under such conditions At physiological doses which presumably more nearly represent the position in the normal animal the liver still plays a part in excretion into the feces via the bile as described above but much less prominently (38) The thyroxine in this case appears to be largely deiodinated during the course of metabolism and the resulting iodide which escapes the highly efficient thyroid trap is excreted in the urine The quantity of iodide excreted in the urine of man thus generally reflects the amount which is not taken up by the thyroid gland

An inverse ratio between thyroid uptake and urinary excretion of iodine has repeatedly been observed in man (88) and has been demonstrated in rats (63) In one experiment with this latter species the elimination of radioiodine into the urine within 24 hours of administration was about 90% in normal animals and 20% in iodine deficient animals The corresponding thyroid uptakes were 8% and 55% respectively (63) In human adults the proportion of an oral dose of labeled iodide excreted in the urine of hyperthyroid patients has been shown to be very much lower than in myxedema cases or in normal individuals This finding is briefly illustrated in Table 38 taken from the publication of Raben and Astwood (88)

TABLE 38

PERCENTAGE OF ORAL DOSE OF LABELLED IODIDE EXCRETED IN THE URINE (88)

	Number of cases	Range of radio iodine excretion in 48 hours	Number of cases	Range of radio-iodine excretion in 24 hours
Normal	15	53–84	23	42–80
Myxedema	6	72–92	10	48–93
Hyperthyroid	26	6–32	34	5–43

It seems therefore that an indirect estimation of thyroid function can be made by determining the urinary excretion of radioiodine after a standard interval of time Such a suggestion was made quite early in the application of radioiodine to the study of thyroid function (51) Urinary estimations of this nature suffer from less drawbacks than the thyroid clearance tests mentioned previously, but they are potentially

inaccurate because they fail to take into account the influence of renal clearance Collecting the urine over three to six successive periods has been shown to eliminate this uncertainty (30) Further the "T" index calculated by Fraser *et al* (30) has been shown to be virtually independent of the renal condition and to be closely correlated with "early thyroid clearance tests in patients with established diagnoses ranging from definite and slight myxedema, to normal and probable and definite thyrotoxicosis This T index or the extra renal disposal rate index

$$\left[\frac{0\text{–}8\text{ hr }\% \times 100}{(8\text{–}24\text{ hr }\%) \times (0\text{–}48\text{ hr }\%)}\right]$$

of Fraser is more sensitive than the crude urinary output especially in the diagnosis of myxedema It is apparent that this radioiodine test is an extremely useful addition to the various laboratory estimations including BMR, plasma protein bound iodine (PBI), and plasma cholesterol determinations which are available for the diagnosis of thyroid dysfunction that cannot be made on clinical grounds alone

V Iodine Requirements

Actual needs or requirements of iodine have been difficult to assess because there is, as yet, no wholly satisfactory method It becomes advisable therefore to outline the methods now used and to estimate their limitations before giving the range of "needs so far found with their aid Four main methods have been used

1 *Methods*

The first of these involves comparisons of the estimated amounts of iodine ingested and excreted by individuals in nongoitrous areas and in areas with varying degrees of goiter incidence The annual iodine intakes in one goitrous and in one practically goiter free area in Switzerland were estimated by von Fellenberg (28) to be 4 7 and 11 4 mg respectively On these figures the daily iodine requirement would be less than 20 μg which in the light of other evidence to be presented appears extraordinarily low This method of assessment can be misleading both because of the great fluctuations in iodine intake from food and water and because of the difficulties associated with delineating borderline states of deficiency Determinations of the average daily urinary losses of iodine are more satisfactory as they provide a particularly good index of iodine intake in a given area Calculations made in this way from urinary losses give a daily iodine requirement for adult humans of between 100 and 200 μg (24)

A second method of arriving at minimum iodine requirements is by means of iodine balance experiments These have been valuable but have given extremely variable results In addition to the difficulties inherent in all balance experiments with the trace elements—such as the small amounts normally retained by the body in comparison with the amounts ingested the influence of variable body stores of the element in question and the difficulty of discriminating between the unabsorbed and the absorbed and re excreted components of the fecal excretion—there are special problems with iodine Excretion of this element occurs to a variable extent through the skin and lungs as well as in the urine and feces These must be taken into account Further the efficiency with which the thyroid salvages iodine from the breakdown of iodine compounds in the body and the amount of iodine released by the destruction of tissue particularly the muscles are important factors determining the net iodine balance Von Fellenbergs early balance studies revealed low retentions and consequently low requirements Scheffer (100) found that equilibrium could be maintained in normal subjects at intakes of 54–155 μg I per day indicating a minimum iodine requirement for iodine balance of about 54 μg Cole and Curtis (22) found that four human adults remained in equilibrium or positive balance when the intake of iodine was 39–162 μg /day Studies made on normal persons maintained at rest in bed under controlled hospital conditions and subsisting on an iodine low diet indicated a basal human adult iodine requirement ranging from 44 to 75 μg daily and averaging 67 μg daily or approximately 1 μg per kg body weight (29) These requirements are comparable with those of Scheffer and those of Cole and Curtis but they were obtained under controlled basal conditions and take no account of individual activity or stresses associated with ordinary existence The question of additional iodine requirements to cope with such factors is considered later

The rate of thyroxine formation and decay has also been used as a means of assessing iodine requirements This method suffers from the serious disability that the extent to which iodine containing end products of thyroxine decay can be reutilized by the thyroid is unknown Plummer and Boothby (85) found that the daily rate of thyroxine decay in human adults ranged from 200 to 400 μg and Thompson and co workers (111) concluded that 300 to 400 μg thyroxine was necessary to maintain a normal basal metabolic rate in myxedematous patients at rest in bed It can be calculated from these results that the amounts of thyroxine supplied to the circulation by the thyroid gland to maintain normal metabolic activity is equivalent to 130–260 μg iodine daily A somewhat

similar technique has been applied to the sheep. The daily thyroid secretion rate in this species was found, by the use of injections of I^{131} and of L-thyroxine, to range from 50 to 500 µg. L-thyroxine daily for different groups of sheep under varying environmental conditions (48). Assuming no reutilization of iodine from the breakdown of thyroxine which is justified only by the absence of specific experimental evidence to the contrary, *this represents an iodine requirement of 32–320 µg. daily.*

A rather more precise, but still somewhat arbitrary, means of assessing minimum iodine requirements is through the use of iodine deficient diets, to which graded amounts of iodine are added. This method has been used with success with rats and chicks for which diets have been devised containing as little as 0.025–0.03 p.p.m. iodine, free from goitrogenic substances and satisfactory in other respects (32, 83). These diets are capable of supporting good growth, but iodine supplementation to give an over all intake of approximately 100 µg./kg. ration (0.1 p.p.m.) is necessary in these species to prevent thyroid enlargement.

2. *Minimum Requirements*

Two criteria may be used in determining minimum iodine requirements, namely, those just necessary to maintain a concentration of 0.1% I in the moisture free thyroid gland or those necessary to prevent any significant enlargement of this gland. Levine and associates have set the minimum requirement of the rat as 0.9 µg. I per day by the first criterion and 1–2 µg./day by the second (64). If these two measures are taken as limits, it can be calculated that the rat requires 20–40 µg. I per 1000 food calories consumed. On the same basis the average human adult consuming 3000 calories daily would require 60–120 µg. I per day. This range includes most of the estimates made from balance experiments but it is lower than the estimate made from a variety of investigations by Elmer (27) who gives the optimal requirement for humans as 100–200 µg. I daily.

Whether Levine's criteria can be applied to species of animals other than the rat and human being is not known. These workers (64) suggest that "until precise data are obtained for different species of animals 20–40 µg./1000 calories of the ration is considered as the minimum iodine requirements of farm animals." Although this statement was made in 1933, precise data are still not available. The fact that goiter does not commonly occur among livestock in many parts of the world where it occurs to a mild but definite degree in the human population suggests a lower requirement per unit of the diet or per kg. body weight for farm stock than for man, but when the very different dietary habits

of these species are taken into account such a deduction becomes exceedingly dubious

A further point of possible importance is that in humans and adult rats energy intake and heat production are nearly the same whereas in farm animals gross energy intake frequently greatly exceeds heat production The requirement for iodine is more properly related to heat production than to energy intake if it is assumed that the destruction of thyroxine is proportional to the metabolic rate it induces Mitchell and McClure (75) have calculated the minimum iodine requirements of various classes of farm stock by applying Levine s standards to estimates of heat production They obtained the following results

Animal	Body weight (lb)	Heat production (cal)	Iodine requirement (μg /day)
Chicken	5	225	4 5–9
Sheep	110	2 500	50–100
Pig	150	4 000	80–160
Cow in milk (40 lb daily)	1 000	20 000	400–800

These estimates of requirements compare extremely well with the minimum consumption figures given by Orr and Leitch (79) for the same species in nongoitrous areas except for the cow It appears that the cow ordinarily consumes very much more iodine than it requires

The iodine requirements of the normal healthy resting adult may be greatly increased in various functional activities and disturbances Strenuous physical exercise fever or infection increase the demand and there is evidence that the requirement is greater in pregnancy and lactation The loss of iodine in milk can result in depletion of the mother s iodine reserve inducing a negative iodine balance which should be compensated for by an increased iodine intake (27) The significant rise in the protein bound iodine of the serum which occurs in human pregnancy has been mentioned earlier Evidence for other species appears to be lacking but it is significant that the most obvious signs of iodine deficiency in farm stock in goitrous areas are the birth of dead or weak calves lambs and foals and of hairless piglets In fact in many such areas the only discernible disability suffered by these animals is that imposed by the stress of pregnancy At the same time it should be pointed out that there is direct experimental evidence that the minimum iodine requirement of the rat is no greater during pregnancy and lactation than it is during growth when both are expressed as the proportion of iodine in the diet necessary to prevent thyroid enlargement (83)

The problem of the requirements for growth compared with those for adult maintenance requires much further study Growing human babies have been stated to need 22–44 μg I daily and older children somewhat more (96) Numerous surveys in goiter areas in different countries have disclosed a much higher incidence of visible goiter in growing children than in adults in the same region

In the above consideration of iodine requirements the nature of the rest of the diet is assumed to be satisfactory This is not always the case in practice High intakes of calcium are known to raise iodine requirements and a marked excess of such elements as arsenic and fluorine for which there is some evidence of iodine antagonism may also affect the position These factors together with a consideration of the significance of various goitrogens in foods, are discussed in appropriate sections later, but it can be stated with confidence that the level of intake of iodine itself is ordinarily of overwhelming importance in determining thyroid function Little is yet known, however, of the forms of iodine in foods in relation to iodine uptake and a great deal remains to be learned of minimum iodine requirements of different species at different ages and in varying functional states

VI Sources of Iodine

Normally the food is by far the major source of iodine in the animal body Iodine is also obtained from the drinking water and from salt Only where the salt has been specially iodized does the intake from this source usually make an appreciable contribution to the daily supplies

1 *Iodine in Water*

Contrary to popular belief the water supply does not contribute important amounts of iodine to either human beings or farm stock except in unusual circumstances The iodine content of the drinking water is nevertheless a frequently used and valuable index of the iodine content of the rocks and soils of a region and hence of its vegetables fruits grains and pastures The iodine content of the water has been correlated with the incidence of goiter in many areas Thus Young *et al* (117) obtained the following figures

Area	Iodine in water (μg/l)	Goiter incidence (%)
Somerset villages	2 9	56
Suffolk villages	8 2	3

Kupzis (60) in Latvia reported the following differences between goitrous and nongoitrous areas

	Iodine in water (μg/l)	
Goiter areas	0.1– 2.0	(12 supplies)
Nongoiter areas	2.0–15.0	(49 supplies)

Many more examples could be cited to illustrate this difference between the potable waters of goitrous and nongoitrous regions An indication of the relative unimportance of the water supply as a source of nutritional iodine can be given from the oft quoted findings of von Fellenberg (28) This worker determined the iodine intake provided by representative foods and by water in an average daily diet in a highly goitrous township and in a goiter free township in Switzerland Von Fellenberg s data are as follows

	μg Iodine in quantities of food stated	
	Chaux de Fonds Goiter free	Signau Goitrous
Bread 300 g	4.9	2.4
Potatoes 500 g	3.5	2.0
Vegetables (mixed) 300 g	4.2	3.1
Milk butter and cheese 1.5 liters (milk equivalent)	13.5	4.5
Apple (or other fruit) 300 g	1.8	0.3
Fat 60 g	0.6	0.6
Water 2 liters	2.8	0.1
Cooking salt 10 g	0	0
Total	31.3	13.0

It will be noted that the drinking water supplies less than 10% of the total daily iodine intake in both areas

2 *Iodine in Foods*

Iodine is greatly concentrated in various forms of marine life especially seaweeds although according to Orr and Leitch (79) sea water itself is not particularly rich in this element containing only 17 to 18 μg/l Figures as high as 0.2% I on the fresh basis have been recorded for some seaweeds Marine plankton is also very rich in iodine but the contribution of this iodine to human and stock populations is largely

indirect, through acting as a source of nutriment to fish Remington *et al* (91) have shown that the effect of marine plankton and sea water, thrown up in the spray, upon the iodine content of soil and crops is negligible, except for a very narrow coastal strip The iodine contents of sea fish and shellfish are high but very variable Monier Williams (76) claims that 400 μg I per kg for sea fish and 900 μg/kg for shellfish are reasonable representative figures for these species The liver oils of many sea fish are exceptionally rich in iodine Levels as high as several hundred parts per million have been reported (46)

All plants contain iodine in amounts ranging from about 2 μg/kg (0 002 p p m) or less, to several hundred times this level Real species differences in iodine concentration appear to exist but the iodine content of land plants depends far more upon the available iodine in the soil than it does upon species Wide variation in the iodine content of the same species grown in different localities is an outstanding feature of available data Attempts to correlate the total iodine content of the soil and that of the plants grown upon it have not been very successful In Table 39 soil iodine and pasture iodine values taken from New Zealand and from England are presented, together with soil pasture iodine ratios These figures which are extracted from the publication of Russell (95) illustrate the great variability which can exist The iodine content of vegetable crops and of pastures can be markedly increased by application of iodides to the soil or by fertilization with manures that are naturally rich in iodine, such as seaweed Chilean nitrate or guano There is some evidence that the effect of such fertilizers is due both to the provision of additional easily accessible iodine and to increased absorption by the plant of iodine already present in the soil (50)

Some idea of the extent of the variation in the iodine content of pasture plants growing under different conditions can be obtained from the data of Table 39 Plant materials used as human food are equally variable in iodine content Thus the following wide ranges of values are quoted in the comprehensive review of Orr and Leitch (79) lettuce 50–620 wheat grain 1–64 water cress 190–450 and nuts 15–200 μg I per kg Monier Williams (76) states that the average" values taken from various parts of the world are 25 μg I per kg for cereals 30 μg/kg for vegetables and 10 μg/kg for fruits The variability from sample to sample, within the same class of foodstuff is so great however that average values have little meaning and *cannot* be applied with any confidence to particular dietaries One generaliza tion that appears permissible is that animal products apart from sea

fish shellfish milk from cows receiving iodine supplemented rations and eggs from hens whose diets have been similarly supplemented are appreciably lower in iodine than vegetable foods

TABLE 39
IODINE IN SOILS AND PASTURES AND THEIR RELATIONSHIP (95)

New Zealand (50)			Derbyshire (79)		
Soil I (p p m)	Pasture I (p p m)	Soil I / Pasture I	Soil I (p p m)	Pasture I (p p m)	Soil I / Pasture I
26 4	0 072	367	3 43	0 79	4 3
20 0	0 048	417	3 58	0 84	4 3
13 0	0 077	169	4 80	0 36	13 3
3 6	0 032	113	8 08	0 30	26 9
3 5	0 024	146	4 93	0 21	23 5
			3 73	0 25	14 9
			7 98	0 35	22 8
			5 23	0 34	15 4

According to Hercus and Roberts (50) who conducted a detailed survey of the iodine content of foodstuffs raised in goitrous and non goitrous districts in New Zealand the foods which contain most iodine are in order of merit fish green vegetables whole cereals milk meat and root vegetables and those which make the greatest contribution to iodine intake in a normal well balanced diet without large amounts of fish are vegetables and milk This claim receives some support from the data of von Fellenberg quoted earlier obtained under different dietary conditions in Switzerland Apart from sea fish and shellfish and such an unusual food as seaweed which does not form a regular part of the diet of most peoples no classes of ordinary foodstuffs are markedly and consistently superior to others in iodine content In contrast to the position with iron and copper it is the *source* of the foods composing a given dietary and not the proportions or choice of such foods which largely determines the over all iodine intake Intensive food consumption surveys carried out in Australia have failed to disclose dietary differences between goitrous and nongoitrous areas (19) or within a mild endemic goiter area between those households with goitrous children and those without (80) At the same time it is neces sary to point out that the food pattern of households at the time of survey may not represent the conditions which prevailed during the period of development of the goiter Nevertheless it can be asserted that the residents of an endemic goiter region cannot normally obtain sufficient iodine for their full requirements by a more judicious choice

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of foods grown within that region Only by the importation of a sub stantial proportion of the food consumed from areas adequately supplied with iodine, or by the general use of iodized salt or the adoption of some such control measure, can the position in such a region be remedied

Japan represents an outstanding example of a goiter free area which is geologically low in iodine whereas Formosa which is similarly low in iodine has a high incidence of goiter among its population The absence of goiter in Japan is believed to result from the consumption of large amounts of iodine rich seaweed which are imported into the country in considerable quantities The Formosans have not developed a similar taste for seaweed or they do not consume it in the same large amounts as the Japanese Equally interesting but less striking, is the position of the inhabitants of the Great Salt Lake area of North America The water of this lake is as high in iodine as that of the ocean (74) and yet, unlike the ocean, it supplies neither fish nor vegetation for human consumption The high incidence of goiter in this area is undoubtedly contributed to by the absence of iodine rich marine foods such as are available on the sea coasts where there is a minimum of endemic goiter

3 *Iodine Supplementation*

Since inadequate dietary intakes of iodine have been established as the primary cause of endemic goiter the obvious method of preventing the disease is by providing additional iodine This has been recognized from the time of the first clear appreciation of the relation of this ele ment to goiter although some of the earlier methods of goiter prophy laxis such as hanging an iodine impregnated wooden pendant from the neck or leaving a few drops of a solution of iodine in a saucer in the bedroom or schoolroom can hardly have been very effective The principal methods of iodine supplementation that have been used and are still used to an extent that depends upon the social and economic conditions of the area are (a) the addition of iodine to municipal water supplies (b) the administration of iodized tablets as candy to school children (c) the fortification of vegetable foods through the application of iodine rich fertilizers or of milk through feeding iodine to the cow and (d) the use of iodized table and cooking salt including the use of such salt in the baking of bread

The first three of these methods are troublesome depend for their success on the cooperation of a number of people or authorities and are rarely completely effective They are not now used a great deal Iodized salt, on the other hand is universally recognized as the most economical convenient and effective way of supplying supplemental

iodine Even this method does not prevent goiter in all cases because some individuals either do not use any salt at all or consume it in insufficient quantities The incorporation of iodized salt into all bread baked appears to be a highly effective means of overcoming this difficulty where as in Western communities bread is a universal item in all diets In some countries such as India and Mexico, where extensive goitrous areas exist programs of iodinization of salt have met with major technical difficulties associated with the problem of producing and distributing dry fortified salt and of maintaining the stability of the added iodide In more highly developed areas of the world these problems have been largely but not yet completely overcome and iodized salt has now been introduced on a community scale under official auspices in many countries Typical data showing the reduction in goiter incidence following the introduction of these measures are given in Table 40

Iodine is usually added to salt either as sodium or potassium iodide or more recently as sodium iodate Potassium iodide has an advantage over sodium iodide in being nonhygroscopic but both iodides possess the disability of being unstable to moisture heat and sunlight If the salt is packed dry and kept dry in an impervious container its iodide content remains fairly stable for many months When these conditions cannot be met the very much more stable iodate should be used

The desirable level of iodinization with iodide or iodate depends upon the daily iodine requirement and the average daily salt intake The latter varies greatly among individuals and among countries In the United States and New Zealand the average consumption of salt per head is only about 6 g daily in European countries it is somewhat higher but in hot humid countries such as southern India it may approach 30 g daily Clearly a lower level of iodinization is necessary in these countries to supply the same average amount of iodine The actual recommended iodide content of salt varies widely In the United States it is 1 part in 10 000 in New Zealand 1 part in 20 000 in England 1 part in 50 000 and in various European countries either 1 part in 100 000 or 1 in 200 000 Fortunately the thyroid gland is capable of adjusting to wide variations in iodine supply and tolerates a considerable excess of iodide so that these wide variations in iodide concentration have little significance The ideal of course would be to iodinate at just that level which would be adequate for the prevention of goiter in an area taking into account the salt habits of the people According to the Endemic Goiter Study Group of the World Health Organization which considered all available evidence the daily intake of iodide necessary for this purpose is 100 μg (103) This allows a certain margin of safety

TABLE 40

Reduction in Goiter Incidence Following the Introduction of Iodized Salt (116)

Place	Year iodized salt introduced	Data apply to	Goiter incidence (%) Before	After
United States				
Michigan State	1924	School children	38 6 in 1923/24	2 9 in 1936
City of Detroit	1924	School children	35 0 in 1924	3 0 in 1930
Cleveland Ohio	1924	School children	31 0 in 1924	7 7 in 1936
Switzerland				
Canton Vaud	1924	Whole population	77 0 in 1924	21 0 in 1937
Canton Appenzell	1922	Recruits	7 0 in 1922	0 1 in 1938
Canton St Gallen	1922	Recruits	4 0 in 1917	0 5 in 1938
City of Lausanne	1924	School children	57 0 in 1924	1 0 in 1937
Poland				
Krakow Province	1935	Recruits	17 6 in 1930/34	2 9 in 1937

and is far below the level which has given any toxic effects On this basis a level of iodinization of 1 part in 10 000 and an average salt consumption of about 6 g daily such as occur in the United States provide a very considerable excess of iodine

The need to apply goiter preventive measures early in life has been emphasized by many writers The earlier iodine prophylaxis is applied the greater its efficacy Iodized salt is highly effective in the prevention of endemic goiter but its curative effect depends upon the character of the goiter as well as on the age of the patient at the time iodine therapy is instituted In patients with colloid goiter iodine administration can be of great value but in older patients with goiters of long standing, in which there are extensive pathological changes little improvement can be expected (27, 79) Prevention is therefore essential in iodine deficient regions by the continued use of iodized salt throughout life but in particular by its commencement during pregnancy and childhood when the demands for iodine are at their greatest

VII Goitrogenic Substances in Food

Dietary factors other than lack of iodine have long been suspected to play some role in the pathogenesis of endemic goiter although it is only within recent years that the nature and mode of action of a number of antithyroid agents some of which occur naturally in foods have been revealed In an overwhelming proportion of cases endemic goiter can be associated with an absolute deficiency of iodine in the food and water supply but cases of nontoxic thyroid enlargement occur sporadically where iodine intakes are well above those found adequate under other conditions and certain diets apparently containing sufficient iodine for normal requirements have been found experimentally to induce histological alterations in the thyroid such as result from absolute iodine deficiency Intensive investigation of such diets has been extremely fruitful in unraveling some of the secrets of the thyroid relating to iodine utilization and have provided a number of compounds of great therapeutic value in the treatment of conditions associated with hyper functioning of the thyroid gland Nevertheless the problem of simple goiter not due to iodine deficiency remains far from solved

1 *Influence of Elements other than Iodine*

Excessively high dietary intakes of arsenic and possibly fluorine may be significant factors in the production of goiter where intakes of iodine are low or marginal Goitrogenic effects from this cause are of rare occurrence they apparently do not occur where iodine intakes are

normal, and they can be prevented or overcome by adequate iodine supplementation This question is considered more fully when dealing with arsenic and fluorine in Chapters 12 and 10 respectively There is a considerable volume of evidence also, that high or relatively high intakes of dietary calcium may be goitrogenic, possibly by reducing iodine assimilation The various findings with calcium are, however, by no means completely concordant and the mechanism by which this dietary factor exerts its goitrogenic effect is not yet clearly established High protein and high fat diets have similarly been associated with the production of goiter (34), but it is the goitrogenic effect of certain vegetable foods which has attracted the most attention and has been of the greatest scientific and practical value

2 *Cabbage and Brassica Seed Goiter*

The first definite evidence of a goitrogen in food was obtained fortuitously by Chesney *et al* (18) who observed the development of large goiters in rabbits fed a ration consisting predominantly of fresh cabbage and who showed that the glandular enlargements could be prevented by a supplement of 7 5 mg iodine per rabbit per week They also noted great individual variability in susceptibility to the cabbage diet and a marked seasonal difference in response These original observations were soon confirmed and extended in different parts of the world and "cabbage goiter" became a popular subject of investigation Within a few years it was shown that other members of the cabbage family or Cruciferae were goitrogenic and that species other than the rabbit were affected Thus Blum (13) found that cabbage was goitrogenic in sheep goats, pigs and geese, giving rise to glands four to six times the normal size and that kale cauliflower, kohlrabi, and turnips were all capable of producing thyroid enlargement in rabbits

During this time attention was turned to the seeds of Brassica species and to the goiter producing possibilities of plants other than members of the Cruciferae family The seeds of cabbage rape mustard, swede" (rutabagas) turnip and chou moellier when fed to young rats were found to produce thyroids three to four times larger than in the controls, even though the iodine intake was adequate (58) Of the greatest importance was the finding that added iodine in contrast to the position with "cabbage goiter had only a moderate or incomplete inhibitory effect on the size of the Brassica seed goiters Evidence was also obtained that a number of other vegetable foods including soybeans peanuts lentils peas and even strawberries pears and carrots may possess a certain degree of antithyroid activity in the rat the chick or

in man (13 35 73 114) Foods reported to be goitrogenic in laboratory animals are given in Table 41 taken from the publication of Astwood (5)

TABLE 41
VEGETABLES FOUND TO BE GOITROGENIC IN LABORATORY ANIMALS

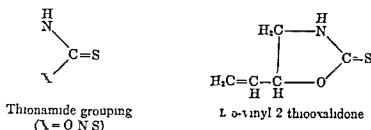
Food	Author	Date
Cabbage	Chesney *et al* (18)	1928
Brussel sprouts and cauliflower	Marine *et al* (70)	1929
Kohlrabi	Stiner (104)	1933
Soybean and peanut	McCarrison (73)	1933
Turnip and seeds of mustard rape and cabbage	Hercus and Purves (49)	1936
Raddish	Indina (54)	1940
Seeds of rutabaga chou moellier soft and hard turnip	Kennedy and Purves (58)	1941
Kale mangel red cabbage lentils and peas	Blum (13)	1942

3 *Nature and Mode of Action of Goitrogens*

Speculation as to the nature and mode of action of the goitrogenic compounds in foods began immediately after the original observation of Chesney and co workers in 1928 but substantial progress was not made until a remarkable series of investigations was undertaken by Kennedy Purves and others in New Zealand in 1941 These workers found that (a) hypophysectomy would prevent the development of goiter in rats fed a rape seed diet (b) small doses of thyroxine would similarly abolish the thyroid hyperplasia (c) iodine would only partially overcome the effects of such diets even when administered in large doses and (d) the thyrotrophin content of the serum of affected animals was as high as that of thyroidectomized animals (37) These findings clearly indicated that rape seed produces thyroid enlargement by interfering with the production of thyroid hormone In this way the pituitary is stimulated to increased thyrotrophin secretion with resulting follicular hyperplasia This continues until the diet is changed or hypophysectomy performed since the thyroid is unable to respond by increased hormone production A similar mechanism has been demonstrated for the artificial antithyroid compounds of the aminobenzene and thionamide series (7)

The chemical nature of the compound or compounds responsible for the goitrogenic effect of Brassica seeds defied scientific attack for a number of years Kennedy (57) no doubt stimulated by the finding

that phenylthiourea would produce hyperemic goiter in rats (92) produced evidence that allylthiourea *was a potent goitrogen and* suggested that it might be the active agent of Brassica seeds This suggestion proved to be incorrect, since thiourea derivatives could not be isolated from such materials Yet these important findings were of the greatest significance as they were directly responsible for the great developments in the *production and study of antithyroid compounds* containing the thionamide grouping including thiouracil during the ensuing decade In 1949 Astwood and his associates (6) isolated and identified the new compound L 5 Vinyl 2 Thiooxalidone from rutabagas and wholly or predominantely accounted for the antithyroid activity of Brassica *seeds by the presence of this compound in a combined* inactive form (36) The structure of this compound and of the thionamide grouping characteristic of the antithyroid compounds mentioned earlier are as follows



Thionamide grouping (X = O N S) L 5-vinyl 2 thiooxalidone

Vinyl thiooxalidone is about one fifth as active as thiouracil and slightly more active than propyl thiouracil in man It has been demonstrated in the edible portion of white turnips kale and rape and in most members of the Brassica family but not in any other family Nor has it yet been detected in the leaves of cabbage This is a curious and so far baffling fact It is possible that the samples of cabbage examined were nongoitrogenic in view of the well established marked variability in the goitrogenicity of cabbage from different localities at different times of the year It seems much more likely, however, that antithyroid compounds other than vinyl thiooxalidone are present in the leaves of cabbage especially as "cabbage goiter can be completely inhibited by iodine *feeding* whereas *Brassica seed goiter due to thiooxalidone* can only very partially be abolished by these means

The isothiocyanates (mustard oils) received early attention as possible goitrogens because they are characteristic constituents of the cabbage family but they were found to be inactive The cyanides were then *investigated Various cyanides* were found to be goitrogenic by some investigators but not by others The most plausible explanation of dis

crepancies of this kind seems to lie in the different animal species upon which they were tried It is probable that the cyanides depend for their antithyroid effect upon conversion to thiocyanate, which has been shown to occur in some species for example rabbits but not in chickens (102) Thiocyanate was subsequently shown to be a goitrogen (8) and to act by inhibiting the selective concentration of iodine by the thyroid Thiocyanate has no measurable effect on the synthesis of thyroxine as thiooxalidone and thiouracil have and induces goiter only when the iodine intake is low or marginal Moreover, its goitrogenic effect like that of cabbage can be completely overcome by the administration of iodine It is doubtful however if cabbage contains sufficient thiocyanate or thiocyanate producing substances to account for its goitrogenic action The problem of cabbage goiter which was responsible for initiating so much outstanding research therefore remains unsolved

4 *Practical Dietary Considerations*

It is necessary to stress that goitrogen containing foods produce goiter only when given persistently and in abnormal amounts Where these foods are consumed in normal quantity and are cooked in the ordinary way goiter is not likely to develop The cooking of vegetables containing thiooxalidone either whole or in chunks prevents any goitrogenesis presumably by destroying the enzyme which liberates this substance from its inactive precursor (36) The body does not appear to contain any enzymes which are capable of so acting although if the vegetable is ground or allowed to stand in water for some hours before cooking it is possible that sufficient hydrolysis might take place to allow appreciable amounts of the active compound to be formed Where the diet is composed wholly or very largely of goitrogenic foods such as turnips or cabbage the amounts of antithyroid material ingested may be sufficient to cause goiter but this occurs only under very abnormal circumstances Examples of such eating habits forced upon certain communities in Europe during the recent war which were accompanied by an increased incidence of goiter have been described (18 34) There is no worth while evidence that the goitrogenic property of members of the Brassica family is of any real significance in the amounts ordinarily present in human diets even vegetarian diets As has been stated (4) "The devotion to cabbage shown by the British people (who have for generations regularly eaten large quantities of the olive-colored soggy mess constituting the less worthy half of two veg) should have provided some evidence if this were so"

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CHAPTER 10

FLUORINE

I Introduction

Fluorine has particular interest as a trace element because of the unique dual role it perform in human nutrition—as the cause of widespread fluorine intoxication and as a preventive factor, in appropriate doses, in the incidence and severity of dental caries All of the essential trace elements exert toxic effects at sufficiently high levels of intake but fluorine is unusual in that the margin between intakes which confer physiological benefit and those which induce toxic symptoms is exceptionally narrow Moreover, in spite of the undoubted benefits conferred upon teeth by fluorine unequivocal evidence that this element is a dietary essential even for dental health, has not yet been produced

For many years, biological attention was almost entirely confined to the toxic aspects of fluorine Chronic endemic fluorosis of man and his domestic animals was shown to occur in certain areas in all five continents and a vast amount of data was accumulated on the fluorine content of rocks, soils, waters and plant and animal tissues and on the dietary and other factors influencing chronic fluorine toxicity A very different set of conditions was revealed from those which operate in endemic selenium or molybdenum poisoning where high concentrations in the plant materials consumed as food represent the main source of the toxic element In endemic fluorosis it is the water supplies, together with surface contamination of plants with fluoritic fumes and dust, that provide the greater part of the toxic intakes by man and beast

The discovery in 1931, that fluorine plays a part in the prevention of human caries which arose from epidemiological studies of "mottled enamel, a manifestation of chronic endemic fluorosis in man gave great additional stimulus to investigations of the physiological significance of fluorine These investigations, including the many collateral studies with laboratory animals, are outlined in the sections which follow

II Fluorine as an Essential Element in Nutrition

No convincing evidence has yet been produced which indicates that fluorine performs any essential function in the nutrition of plants or microorganisms Long before the present interest in this element in relation to dental caries arose, the view was fairly widely held that it

was an essential element in animal nutrition concerned especially with the formation and structure of teeth This view seems to have arisen by teleological reasoning from the constant presence of fluorine in the bones and teeth as well as in other tissues and organs notably the thyroid and epidermal structures This was shown by Gay Lussac and Bertollet as long ago as 1805 and has been amply confirmed with the more sensitive and reliable methods of estimation developed since that time Numerous attempts to demonstrate directly an essential function for fluorine by the use of fluorine low diets have nevertheless not been completely successful

Sharpless and McCollum (93) obtained just as good growth and reproduction with no abnormalities in tooth and bone structure or composition other than low fluorine content in rats fed a highly purified low fluorine diet as in rats on the same diet to which 10 p p m fluorine had been added Evans and Phillips (21) found that a mineralized milk diet containing 1 6 p p m F on the dry basis and supplying only 2 μg or about 50 μg per kg body weight daily was adequate for the growth general well being and reproduction of rats through five generations The bones and teeth were strong smooth evenly and well calcified and there was *no depletion of fluorine stores or increase in demand for* this element throughout the five generations Moreover additional fluorine as graded doses of sodium fluoride produced no discernible improvement in the animals Lawrenz (44) carried 14 pairs of rats through a feeding period of 207 days on diets containing 0 47 and 2 5 p p m F on the air dry basis The rats were produced from mothers that had themselves subsisted on a low fluorine diet and they contained only 0 8 p p m fluorine at birth compared with 5–7 p p m in the bodies of young produced on a stock diet of natural foods No significant differences in the rate of growth or the dry weight of the skeleton or teeth were exhibited by the rats on the two diets and no signs of malnutrition or abnormalities in the teeth of those on the fluorine low basal diet were observed These findings do not of course prove that fluorine is a dispensable element in the nutrition of this species but they do indicate that if required at all the minimum requirement must be less than 0 5 p p m of the diet

A new approach to the problem has been offered by McClendon and Gershon Cohen (54) These workers describe a method for growing green feedstuffs and yeast in water culture made "fluorine free" A basal diet of materials produced in this way from which fluorine was claimed to be absent was fed to weanling rats for 66 days The same diet grown in the same way but with added fluorine was fed to con

trol animals for a similar period The control animals averaged 128 1 g live weight and 0 5 carious molars per rat at the end of the experiment, whereas those on the fluorine free diet averaged only 51 2 g live weight and had 10 2 carious molars per rat at this time These differences are so great that they can hardly have been due to chance but the whole experiment would be more convincing if actual fluorine analyses of the diets and of the animals had been carried out and presented The possibility that the differences between the groups were due to factors other than fluorine cannot entirely be excluded Nevertheless the approach used by these workers is extremely interesting and might well be extended to include a number of other trace elements

III Chronic Fluorosis of Livestock

1 *Sources of Fluorine*

The ubiquitous occurrence of fluorine in soils plants and drinking waters ensures a continuous intake of this element by all animal species The amounts ingested from these sources are normally too small to constitute a serious health hazard Pastures and hay crops usually contain no more than 1–2 p p m F on the dry basis although levels as high as 9–13 p p m have been recorded (52) and similar levels in grasses watered by highly fluorided water in Queensland have been observed (38) The cereal grains may contain up to 3 p p m F but levels of 1 p p m or less, are more common (55) These concentrations reflect the limited ability of most plant species to absorb this element from the soil even where fluorine containing fertilizers are applied The contrast in this respect to the position in endemic selenium poisoning where very high concentrations in the herbage of affected areas are characteristic has already been mentioned

Surface supplies of drinking water for domestic or stock usage also normally contain inappreciable quantities of fluorine even in rock phosphate regions like Morocco or Tennessee or in areas characterized by high phosphate soils (52) Intakes of fluorine sufficient to induce chronic endemic fluorosis are thus confined to particular regions where the waters are naturally high in fluorine or where the herbage is contaminated with phosphatic rock dusts or with emanations from certain industrial plants or volcanic eruptions The addition of fluorine bearing minerals as calcium and phosphorus supplements to farm rations may also result in excessive ingestion of fluorine It was in fact the use of rock phosphate in this way which provided the first evidence of a fluorine problem in ordinary feeding practice

The problem of fluorine intoxication from industrial sources lies rather outside the scope of this text It has been thoroughly discussed by Roholm (69) in his classical monograph on this subject and more recently by Agate and co workers (3) The relation of fluorosis to emanations from volcanic eruptions is of special interest because this source provided the earliest known records of fluorosis in either man or animals (89) The other sources of fluorine that have been mentioned are however of much greater general significance to the problem of chronic fluorine poisoning

For many years a disease of cattle sheep horses and man known as "darmous has been recognized in parts of North Africa This disease was shown by Velu (103) to be due to fluorine poisoning consequent upon the contamination of the herbage and the water supplies with phosphatic dusts blown from the phosphate deposits and mines of the area Neither the herbage plants nor the water supplies are *inherently* high in fluorine despite the phosphatic character of the soils It is the continuous physical contamination of the environment with fluorine containing rock dusts which supplies an overwhelming proportion of the fluorine ingested (27)

North African and North American rock phosphates invariably contain 3–4% fluorine and products made from them such as superphosphate and dicalcium phosphate retain a varying proportion of this fluorine of the order of one quarter or less Special defluorinating processes have been developed for these materials in recent years but ground rock phosphate from the above sources can definitely be injurious to stock in the amounts ordinarily employed as mineral supplements to farm rations (80) No such evidence exists for rock phosphate and its products obtained from the Pacific and Indian Ocean island deposits which supply phosphate to Australia and New Zealand (98a) These rock phosphates contain on the average only 1 5–2 5% fluorine depending upon their source (98a)

In North America fluorine-containing phosphatic supplements are a sufficiently serious source of fluorine to farm animals to warrant control measures limiting their fluorine content The following tentative regulation has been promulgated in the United States

"The fluorine content of any mineral or mineral mixtures that are to be used for the feeding of domestic animals shall not exceed 0 30% for cattle 0 35% for sheep 0 4% for swine and 0 6% for poultry"

The remaining major source of fluorine is the water supply This is easily the most important and widespread source of toxic quantities of this element Chronic endemic fluorosis of sheep and cattle from this

cause occurs over fairly extensive areas in the United States and many other parts of the world. High fluorine waters are usually from deep wells or bores in which the fluorine comes not from leachings from surface zones but from deep seated rock formations. This applies to the deep wells supplying fluorided water in Texas and to the thermal bicarbonate artesian bore waters of Queensland. The levels of fluorine in these waters are very variable but original concentrations of 2–5 p p m are most common, 10–14 p p m occur not infrequently, and where evaporation has taken place in troughs and bore drains levels as high as 40 p p m have been recorded (38). Since the water consumption of grown sheep in these areas approximates 4–5 gallons weekly, or about 2 5–3 kg daily, it will be seen that the fluorine intake from such waters can be quite high. At 5 p p m it will amount to 12 5–15 mg daily and at 10 p p m, to 25–30 mg daily.

2 *Manifestations of Chronic Fluorosis in Livestock*

Observations of animals suffering from endemic fluorosis and from experimentally induced fluorosis have enabled the symptoms of chronic fluorine poisoning to be clearly delineated. For some time, which may amount to a year or more in herbivorae, no ill effects can be discerned from the continuous ingestion of amounts of fluorine that eventually prove seriously toxic (75). This is due to the buffering effect of the skeleton, in which most of the ingested fluorine retained by the animal is deposited. The fluorine content of the bones and teeth increases in proportion to the amount, duration, and continuity of intake (23), and the store of fluorine in the skeleton can increase to ten times that of normal animals before morphological changes in the bones appear (see Table 42). This immobilization of the greater part of the absorbed fluorine continues until a saturation state is reached. The absorbed fluorine is then free to exert general toxic effects, rapid metabolic break down occurs, and death ensues.

Before this stage is reached various clinical manifestations of chronic fluorosis become apparent. The teeth become modified in color, shape, size, orientation, and structure. The incisors become pitted, the molars abraded, and in some cases there is exposure of the pulp cavities due to fracture or wear. Well defined exostoses develop in the jaw bones and long bones, and the joints become thickened, making movement difficult and painful. It must be emphasized that these dental abnormalities occur only in animals exposed to excess fluorine ingestion prior to the eruption of the permanent teeth—mottling of the teeth in farm stock

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as in human beings is not observed when the high fluorine intake begins after the deposition of the enamel

TABLE 42
THE MEAN FLUORINE CONTENT OF THE ORGANS AND TISSUES OF SHEEP[a] (38)

Tissue or organ	Normal sheep (2 sheep)	Sheep consuming water containing 5 p p m F for a period of 2 years (14 sheep)	Sheep consuming water containing 10 p p m F for a period of 2 years (14 sheep)
Liver	3 5	1 9(1 0– 3 2)	2 4(1 0– 8 4)
Kidney	4 2	12 3(0 2–26 2)	20 0(5 4–43 4)
Thyroid	3 0	6 8(4 6–10 0)	7 6(6 0–13 0)
Pancreas	2 8	2 0(1 0– 3 0)	3 2(1 5– 5 2)
Heart	3 0	2 0(1 0– 2 4)	2 3(1 0– 6 0)
Muscle (gastrocnemius)	2 0	1 9(1 0– 3 0)	2 2(1 0– 5.5)
Wool	–	7 1(4 6–11 6)	7 6(4 4–12 4)
Femur	225	1222	1931
Tibia	200	829	1322
Mandible	220	1610	2177
2nd incisor	86	903	1231
2nd molar	100	656	980
Dentine	*210*	*1494*	*1847*
Enamel	60	613	754

[a] Measured in p p m F on dry matter of soft tissues and on dry fat free bony tissues

The gross dental lesions and bone and joint abnormalities may severely limit the animals capacity to gather and masticate fodder in the field Anorexia and unthriftiness are general growth is poor and loss of weight and emaciation occur in mature animals Wool production is reduced in sheep and milk production in cattle These arise indirectly as a result of and in proportion to the reduction in food consumption There appears to be no specific functional impairment of these processes or of reproduction in the fluorotic animals except in severely affected cases The poor lamb and calf crops that characterize flocks and herds in endemic fluorosis areas are the result of mortality of the young at or near birth due to the impoverished condition of the mother rather than to a failure of the reproductive process itself There is little or no placental transfer of fluorine from the mother to the fetus and no appreciable amount of fluorine is transmitted through the milk (38) Newborn and suckling animals do not therefore exhibit the symptoms of chronic fluorosis in endemic fluorosis areas in the same way that new

born and suckling animals are affected with selenium poisoning in endemic seleniferous areas

In horses (103) and in pigs (42) clinical signs of chronic fluorosis similar to those just described for sheep and cattle have been reported Such symptoms have also been produced in small laboratory animals by fluorine feeding In rats and puppies anemia occurs as an additional symptom, owing presumably, to the bone lesions extending into the marrow, so reducing its capacity for blood regeneration (8) Anemia is not a conspicuous feature of chronic fluorosis in farm stock

These clinical symptoms of chronic fluorine poisoning are insufficient of themselves, to permit a secure diagnosis For this recourse must be made to fluorine analyses of tissues Franklin (25) has drawn attention to the close similarity in sheep between the dental abnormalities resulting from calcium deficiency and calcium phosphorus imbalance and those occurring in chronic fluorosis the similarity in cattle between the syndrome of osteomalacia and that of chronic fluorosis has been pointed out by Indian workers (53a) If fluorosis is acquired during the period of tooth formation the condition of mottled enamel is specific but if exposure to fluorine occurs after the teeth have erupted mottling *does not occur although the erupted teeth may acquire additional* fluorine

The most reliable criterion of fluorosis is the fluorine content of the bones and teeth This of course can normally only be obtained *post mortem* Fluorine estimations on the blood and urine of the living animal *also have some diagnostic value provided it is appreciated that these* give an estimate of the contemporary level of ingestion or excretion of fluorine and are not necessarily evidence of the existence of fluorosis Thus Majumdar *et al* (53a) observed a two to four fold increase in the fluorine content of the blood of cattle receiving 160 p p m fluorine in their ration, *the rise being from 1 μg/ml blood to 3 7–4 6 μg/ml It is* of particular interest that this rise occurred within two months or less of the commencement of fluorine feeding before there was any clinical evidence of fluorosis

A direct correlation between the fluorine content of the urine and the concentration in the drinking water has been demonstrated in man (56) and the use of the fluorine concentration in the urine has been recommended as a means of diagnosing subclinical fluorosis in cattle (9) Presumably this would apply also in sheep although such a relationship does not seem to have been studied in this species In advanced fluorosis in cattle urinary fluorine concentrations of 16–68 p p m have been reported compared with normal concentrations of 2–6 p p m Such

high levels may persist in the urine for many months after removal of the animals from the source of the fluorine because of slow skeletal decontamination

3 *Minimum Harmful Levels of Fluorine*

The minimum levels of fluorine intake from the food and water supply which will produce harmful effects on the animal or the maximum levels compatible with their continued growth and well being depend upon a number of factors The most significant of these are the species the chemical form in which the fluorine is ingested the duration and continuity of the intake and the nature of the rest of the diet The number of experiments in which all of these factors are controlled or are comparable, are so few (except for the albino rat) that precise statements of the maximum safe or the minimum deleterious intakes of fluorine are not possible for farm animals For human beings as is shown later the minimum toxic levels are better defined

a *Species Differences* All workers are in agreement that poultry are much less susceptible to chronic fluorine poisoning than are mammals Species differences among mammals are of doubtful significance A maximum innocuous level of 0 032–0 042% fluorine as rock phosphate for growing chickens and of 0 053–0 070% for laying hens has been reported (28 32) The borderline toxicity level of fluorine as rock phosphate for cattle sheep and pigs is about 0 01% or 100 p p m of the total dry ration (68) This level of intake may be associated with some damage to dental structure in sheep and cattle if continued from an early age for several years but there is no effect upon appetite general health or reproduction in females The relatively high fluorine tolerance of poultry compared with that of mammals is due very largely to poorer absorption of this element from the digestive tract From the results of intraperitoneal injections of fluoride it seems that more effective elimination may also be a factor of some importance in these species (36 77) A species difference in the susceptibility of the enzyme systems involved in cellular metabolism has not been demonstrated but cannot be excluded from consideration

When the fluorine is ingested from fluorided drinking water toxic effects are produced at much lower intakes than these Domestic animals are less susceptible to water borne fluorides than man but there is considerable disagreement in the reported minimum toxic levels Mottled enamel has been observed in cows limited to drinking water containing 4 5 p p m F (13) the consumption of water containing 7 or 8 p p m F has been reported to induce no visible effects upon the teeth of sheep

goats, cows and donkeys (102, 104), water containing 2 p.p… been stated to produce lesions in the teeth of lambs (70), and… bore water containing 5 p.p.m. F has been shown to bring abou… dental abnormalities in sheep and to affect adversely appetite… and wool production (38). These apparently discordant findi… almost certainly be explained in terms of environmental diffe… such as atmospheric temperature and succulence of feed, whic… greatly influence the volume of water consumed and of variatio… the field in the duration of access to and protection from fluo… waters. The extent of evaporative concentration of these waters, the… of animals when first exposed to the fluorine, and the nature and… position of the feed, including its fluorine content, may also be impor… factors.

Additional light has been thrown upon this problem by the carefu… controlled pen feeding experiments of Peirce (76). This worker com… pared the effects over a period of three and one half years of wat… containing 2.5, 5, 10 and 20 p.p.m. F (as sodium fluoride) upon shee… fed an adequate diet composed of chaffed hay and crushed grain. The… sheep were 10–11 months of age at the beginning of the experiment… at which time none of their permanent incisors had erupted. No adverse effects of any sort were observed in the animals exposed to unrestricted consumption of the water containing 2.5 p.p.m. F. A slight degree of mottling of the incisors of some of the sheep consuming the 5 p.p.m. F water was noted, but there was no effect on appetite or growth. The water containing 10 p.p.m. F induced a much greater degree of mottling of the incisors and selective abrasion of the molars but no other adverse effects. Marked dental lesions were apparent in the sheep consuming the water containing 20 p.p.m. F but again there was no significant reduction in food consumption or wool growth. In a further experiment with *mature* sheep grazing sown pastures, Peirce demonstrated that the consumption of water containing up to 20 p.p.m. F for a period of 26 months, had no deleterious effect on the teeth or on body weight, wool production or general health, although the concentration of fluorine in the bones and teeth was up to three times that of control sheep.

It should be appreciated that the type of ration used by Peirce was such that practically no initial biting by the incisors would be necessary and little mastication by the molars. Under these conditions it would be expected that the dental defects would of themselves impose no hard slip on the animal. The position may be very different in the field. Much of the available feed in endemic fluorosis regions is harsh and fibrous, causing severe wear of both incisors and molar teeth and fre…

tures along lines of weakness The dental lesions of fluorotic animals then limit food consumption and utilization and constitute the main reason for the impaired growth and wool production characteristic of endemic fluorosis in sheep Such effects have been reported in sheep in Queensland not only where the water contains 10 p p m F or more but as mentioned above where it contains 5 p p m F At this latter level the dental lesions obtained in the pen feeding experiments just described were very slight

b *Relative Toxicity of Different Fluorine Compounds* The critical levels of fluorine in the ration referred to at the beginning of the preceding section relate only to rock phosphate These levels would need to be greatly reduced if the source of fluorine were the highly soluble sodium fluoride Conversely they would have to be greatly increased if the fluorine was in the form of the relatively insoluble calcium fluoride On present evidence it seems that cryolite and rock phosphate are roughly equivalent in toxicity per unit of fluorine

The relative toxicities are not constant but vary greatly with the amounts fed such that the lower the amounts fed the less the variation in toxicity Mitchell and Edman (68) give the following comparison between sodium fluoride and rock phosphate as they affect the rat

Fluorine as rock phosphate (p p m)	Toxic equivalent of F as sodium fluoride (p p m)
14	14
100	50
600	200
36 000	900

At the levels of fluorine of significance in human nutrition differences in toxicity relatable to the chemical form of the fluorine are probably of little importance In sheep and cattle however the fact that the toxicity of fluorided waters is greater than that of the same daily fluorine intake from rock phosphate is at least partly due to the difference in the chemical form In artesian waters the fluorine presumably comes from parent minerals as calcium fluoride but in these waters the element occurs as a much more soluble fluorine salt or salts probably as a result of reactions involving bicarbonate at high temperatures and pressures (38) A toxicity of the more soluble inorganic compound of fluorine higher than that of the less soluble is to be expected when they are ingested in amounts above the solvent capacity of the animal's digestive fluids Further the manner in which the fluorine is administered affects

the assimilation and hence the toxicity of different fluorine compounds At low levels the fluorine of sodium fluoride and of cryolite was found to be about 20% more assimilated by the rat when administered in the drinking water than when consumed in the same amounts in the solid state in the food

c *Duration and Continuity of Intake* The very considerable capacity of the bones and teeth to immobilize fluorine before morphological changes appear in these structures makes it possible for animals to ingest, with perfect safety, amounts of fluorine which eventually prove seriously toxic The length of time over which such amounts of fluorine can be tolerated depend primarily upon the species, the level of intake, and the nature of the fluorine compound, as has been pointed out It depends also on the continuity of intake The fluorine deposited in the skeleton is not stable but is in dynamic equilibrium with the body tissues and fluids so that during periods of low fluorine intake the skeletal stores are slowly depleted providing an opportunity for further immobilization of fluorine during any subsequent period of high intake The physiological effects of intermittent dosage of a given level of fluorine should therefore be less than those produced from continuous dosage

This has been convincingly demonstrated in the growing rat, using synthetic cryolite as the source of fluorine (47), and has been shown by Harvey (39) to be a factor of the greatest practical importance in sheep in the endemic fluorosis areas of Queensland This worker compared the effects on three months old lambs of continuous exposure for 30 months to water containing 10 p p m F of alternate exposure to and protection from such water for three monthly periods and for six monthly periods and of alternate exposure for six months and protection for three months The lesions of fluorosis were apparent in the incisor teeth of all sheep but they were minimal in the group alternately exposed to and protected from fluorine at three monthly intervals The fluorine concentrations in the bones and teeth were also least in this group These findings offer a practical means of at least a partial protection of stock in fluorosis areas where some surface water can be provided especially as the concentrations of fluorine in the naturally fluorided water in the field are generally lower than the 10 p p m employed by Harvey

d *The Effect of the Composition of the Diet* The effect of other dietary components on the toxicity of fluorine has been studied largely with the rat The mitigating effect of calcium salts has been demonstrated in this species by several groups of workers Lawrenz and

itchell (45) showed that with diets containing 9–10 p p m F an crease in the dietary concentration of calcium from 0 23% to 0 73% hosphorus remaining constant) depressed the total retention of fluo ie by 10 to 13% in growing rats and to a greater extent the deposition fluorine in the teeth and soft tissues Increasing the phosphorus from 4% to 0 71% while the calcium remained constant did not appreciably odify the fluorine retention Raganathan (84) claimed that calcium tate gluconate phosphate oxide carbonate and chloride were equally ective in mitigating the toxic effects of fluorine in rats and that the lts of magnesium offer some protection but those of barium and uminum offer none

This finding with respect to aluminum is not in accordance with the sults obtained by other workers Sharpless (92) noted a marked eviating effect of aluminum trichloride on the toxicity of sodium oride in rats Similar favorable effects from aluminum salts have been ported by others with this species (105) and with cattle (53) From ineral balance studies conducted with sheep Becker and co workers 05) conclude that the aluminum functions by reducing the absorption fluorine from the intestinal tract Apparently calcium salts function the same way although Harvey (38) found that raising the calcium ncentration in the diet from 0 2% to 0 8% with calcium carbonate, or lfate or with bonemeal was completely ineffective in combating iorosis in sheep on waters containing 5 or 10 p p m F This worker und further that supplementing the low protein basal diet of cereal iaff with a protein concentrate was equally ineffective although it duced better growth in the animals

Fluorine in Animal Tissues and Fluids

The universal occurrence of fluorine in low concentrations in soils and ods ensures its continual ingestion by animals and its presence has en demonstrated in all animal tissues and fluids Under normal condi ons where the intakes are small an equilibrium exists between the nounts assimilated and the amounts excreted so that there is little cumulation within the tissues The soft tissues have little affinity for iorine and rarely contain more than 2–4 p p m on the dry basis in the ormal animal. The bones and teeth avidly combine with absorbed iorine as would be expected from the known affinity of apatite for is element and the probable apatite structure of the main mineral omponent of these tissues Normal concentrations of fluorine in the ones are 100–200 p p m on the dry fat free basis with usually some hat lower levels in the teeth

In endemic fluorosis areas or under conditions of abnormally high intakes of fluorine with the food, the avidity of the bones and teeth for fluorine and its ready absorption from the alimentary tract result in concentrations in these tissues many times those of normal animals. Levels of 1000–2000 p.p.m., or more, are common where the animal has been exposed to high fluorine intakes continuously for long periods. The concentrations of fluorine in the bones generally respond to an increase fluorine intake to a greater degree than do these concentrations in the teeth and within the teeth the dentin to a greater degree than the enamel. The fluorine content of the soft tissues is also correlated to a small extent with that of the diet but there is no appreciable accumulation of fluorine in any of these tissues with the exception of the thyroid and the kidneys. Even in these organs the concentrations remain very low by comparison with those of the bones and teeth.

The edible portions (muscle and organ meats) of animals suffering from fluorine poisoning do not therefore contain sufficient fluorine to constitute a health hazard to man. These points are illustrated in Table 42 in which figures for the fluorine content of the organs and tissues of normal sheep and of sheep consuming fluorided water continuously for a period of two years are presented. These are taken from the extensive investigations of Harvey (38).

The fluorine content of the milk of normal cows and ewes averages 0.2–0.4 p.p.m. Studies of the effect of high fluorine intakes in the food and drinking water on the fluorine content of the milk of these species have given conflicting results but the most acceptable data indicate that the changes are very slight and of no practical importance. Thus Phillips *et al.* (81) report values of 0.25–0.5 p.p.m. for the milk of cows ingesting rock phosphate. These values are comparable with those of normal animals. Harvey (38) found that the milk of cows from endemic fluorosis areas in Queensland did not exceed 0.25 p.p.m. F and that the milk of ewes consuming water up to 10 p.p.m. F did not exceed 0.2 p.p.m. Even in an area in which the pasture was heavily contaminated with fluorine from industrial sources the highest values were found to be 0.44 p.p.m. F for cows milk and 0.62 p.p.m. for ewes milk (3).

At very high fluorine intakes there is evidence that placental transfer of fluorine to the fetus may be sufficient to affect the newborn animal but at the intakes common in endemic fluorosis areas or usual from mineral supplements containing rock phosphate fetal transmission of this element is too small to have harmful results (38). As was pointed out earlier this is of considerable practical importance. Unlike animals

in endemic selenosis areas newborn lambs and calves in endemic fluorosis areas do not exhibit any symptoms of poisoning and do not begin to acquire excess fluorine until they commence consuming fluoride contaminated food or water There is therefore no advantage in weaning early but transfer to fluorine free water and food before the young animal begins to supplement its mother s milk with appreciable quantities of water and food is clearly advisable wherever possible in endemic fluorosis regions

Unlike milk eggs may acquire significant amounts of fluorine from hens consuming high fluorine rations most of which is deposited in the yolk probably in combination with the complex yolk lipids Fluorine occurs in the yolk of eggs from hens fed normal fluorine low diets to the extent of about 0 8–0 9 p p m (78) The concentration in the yolk may be increased to an average of 3 p p m when the hen s diet is supplemented with 2% rock phosphate so that it contains 0 07% F (78) Similar increases were obtained by the intravenous injection of 0 03 mg sodium fluoride into hens every fifth day (83) but another group of workers found no more fluorine in the eggs of hens subsisting on a ration containing 2 9% rock phosphate than when the hen s ration contained 2% bonemeal (28) It should be noted that the fluorine values reported in this latter study were very much lower than those obtained in the experiment with rock phosphate quoted earlier

IV Fluorine in Human Nutrition

1 *Normal Fluorine Intakes*

Estimates of the average daily intake of fluorine by adult humans in areas affording no unusual exposure from industrial sources or from fluoride bearing waters range from under 0 5 mg to over 1 mg (50 51) The principal factors determining the magnitude of the intake are the fluorine content of the local water supply and its level of consumption and the amounts of tea and of sea foods included in the dietary In infants certain prepared baby foods high in powdered bone may also constitute an exceedingly important source of fluorine (34)

The emphasis upon water supplies in public health studies on mottled enamel and caries prevention in which the water contains about 1 p p m or more of fluorine has tended to create the impression that the drinking water is the principal normal source of fluorine in human nutrition For the overwhelming proportion of mankind the food and not the water is the main source of this element in the diet An average daily intake of 1200–1500 ml of water containing 1 p p m F would supply adults with 1 2–1 5 mg F which is more than would normally be ingested in

the food. But most domestic water supplies contain appreciably less than 1 p.p.m. F, unless artificially fluoridated. Concentrations ranging from a trace to 0.2 p.p.m. have, in fact, been reported for the natural domestic water supplies of most large cities where the position has been investigated. Such waters would, of course, contribute relatively small amounts of fluorine to the daily diet.

Very few foods, with the exception of sea fish and fish products, contain more than 1 p.p.m. F and most of them contain less than 0.5 p.p.m. (57). The quality of the diet, in ordinary nutritional terms, has therefore little direct influence on the magnitude of the fluorine intake, although the proportion of particular nutrients, notably calcium, may affect the assimilation of ingested fluorine. Sea fish and fish products, such as fish paste, contain much higher concentrations of fluorine, of the order of 5–10 p.p.m. McClure (57) has estimated that 50 g of canned sardine may provide 0.8 mg fluorine. The very high fish diet of the inhabitants of Tristan da Cunha, where the water contains only 0.2 p.p.m. F, has been suggested as the cause of the relatively high incidence of mottled teeth found in that population (99).

In many communities tea is the dietary item of the greatest significance in determining the level of fluorine intake. Fluorine concentrations of 100 p.p.m. or more in tea, especially China tea, about two thirds of which passes into the infusion, are not uncommon (33, 37). One cup of tea may increase the fluorine of the diet by 0.2 mg (37), so that under English and Australian dietary habits 1 mg of fluorine daily can be ingested by adults from this source alone. In the balance studies of Ham and Smith (35) it was found that the average fluorine intake of three young women rose from 429–792 μg daily when the subjects were consuming a normal (tea-free) diet to 1200–1368 μg daily when this diet included 1360–1815 ml of tea infusion per day.

The only other normal food items likely to contribute major amounts of fluorine to human dietaries are those containing bonemeal or other fluorine-bearing phosphates, such as the acid calcium phosphate used in baking powders which may be made from rock phosphate. These materials are not usually consumed in large amounts in adult diets but their capacity to increase fluorine intakes greatly was demonstrated in the experiment of Ham and Smith (34, 35) mentioned above. The average fluorine intake of young women on a normal diet was found to rise from 429–792 μg daily to 946–1435 μg daily when this diet was supplemented with 70 g/day of a proprietary baby food containing powdered beef bone and carrying 11–12 p.p.m. F. Such products can contribute the major part of the fluorine ingested by infants, so much

so that their inclusion may raise the level of fluorine in the daily diet of four months old infants to as high or higher (407–706 μg) than that in the much larger amounts of food consumed by adults (34)

The fluorine content of edible foods grown in areas where the local water is above normal in fluorine content or where phosphatic fertilizers are used is not appreciably greater than normal but small increments of fluorine may be added to certain foods when they are cooked in fluoride waters (98) Nor as was mentioned previously is the fluorine content of milk significantly increased beyond normal levels by high fluorine intakes The increased fluorine content of eggs from fluorine fed hens would not greatly influence fluorine intakes from most diets

2 *Fluorine Retention*

Evidence from limited studies of human bones indicates some retention of fluorine from normal diets during life Glock *et al* (29) found the fluorine content of the fat free rib bones of children up to 15 years to be 0 02–0 08% and that of adults 22–68 years of age to be 0 06–0 31% None of these individuals had been exposed to abnormal amounts of fluorine Samples of the foot bones of three individuals aged 17–23 years were shown in a later study (35) to contain 0 016–0 023% F compared with the very much higher figures of 0 10–0 24% for similar bones of three individuals aged 78–82 years These findings suggest that human beings are able to retain fluorine from ordinary intakes but they do not show whether such retention is continuous or constant throughout life Considerable experimental evidence obtained with the rat indicates clearly that this species has a much greater capacity to retain fluorine during growth than at maturity The growing rat can accumulate fluorine in its bones and teeth at a comparatively rapid rate during growth but reaches a relatively constant level of skeletal fluorine at maturity This is maintained throughout the remaining years of life so long as the fluorine intake remains stabilized (48 110) A similar relation between age and fluorine retention in the human species receives some support from balance studies mentioned below but many more studies with individuals of different age groups must be carried out before this can be established with certainty

Fluorine balance studies with human adults are somewhat at variance on the question of the levels of intake at which appreciable retention occurs McClure and co workers (65) maintain that the adult human body eliminates the major portion of food and water borne fluorine with essentially no retention when the quantities ingested are less than 4–5 mg daily In their experiments body sweat was found to contain

appreciable quantities of fluorine Machle and co workers (50) found the input and output of fluorine at low levels of intake (about 0 5 mg daily) to be equal over many weeks with a maximum deviation from equilibrium of ± 10% At levels of intake ranging from 3 to 36 mg/day, definite retention was found in all instances the magnitude of which depended upon the fluorine compound tested and upon the amount of fluorine absorbed (the amount ingested minus the amount in the feces) (49) Ham and Smith (35), on the other hand, demonstrated considerable (31–54%) retention in young women at intakes of only 429–792 μg fluorine daily It should be pointed out however that the periods were very short and the numbers of subjects small Also no account was taken of possible losses in the sweat, although any such losses were probably small since the experiments were not conducted during the summer months Even in infants on milk formula diets slight retention of fluorine has been demonstrated with greatly increased retention upon inclusion of a baby food containing bonemeal (34)

Some of these apparent discrepancies undoubtedly reflect differences in absorption of fluorine due to variations in the sources of fluorine and in the nature of the rest of the diet Ordinary food fluorine is apparently well absorbed and excreted mainly in the urine so that fecal fluorine can be regarded largely as the unabsorbed portion of the intake As the intake rises from normal low levels more fluorine tends to b- absorbed and excreted but more is retained According to Machle a Largent (49) a daily urinary output in adults of 10 to 20 mg fluori is consistent with good health for periods of at least five to seve years The absorption of added fluorine depends on the aqueou solubility of the fluoride salt and its state (i e whether in the drinkin water or as a solid in the food) at the time of absorption (49) Th fluorine in bonemeal is relatively poorly absorbed by human adults whereas that in tea, which is water soluble and not in association with calcium to any degree is well absorbed This has been demonstrated in growing rats (46 86) and in human adults (35) The difference between bonemeal and tea in this respect is illustrated by the experiments on young women cited above If the presumption is made that fecal fluorine is unabsorbed fluorine, the percentage absorption averaged 84% on the tea diet and 55% on the diet which included the baby food containing bonemeal yet the levels of fluorine ingestion were similar in each case On the other hand the percentage of absorbed fluorine which was retained was greatest when the diet included bonemeal and least when tea was ingested This may, of course have been simply due to the smaller amount of fluorine absorbed from the bonemeal diet

3 *Fluorine and Dental Caries*

a *The Human Caries Fluorine Relationship* The first suggestion of a relationship between the fluoride content of domestic waters and the incidence of caries came as a result of the remarkable series of epidemiological investigations begun in Colorado in 1908 followed by studies of the effects of changed water supply and of fluorine ingestion in laboratory animals which revealed the effect of excessive quantities of fluorine in the drinking water upon the incidence of mottled enamel in man Incidental to these studies it was noted that dental caries incidence was highest in communities whose water supply was free from fluorides and approached a minimum in communities whose water supplies contained approximately 1 0–1 5 p p m F Appreciable mottling does not normally occur at these concentrations which have come to be regarded as optimal (20)

The existence of a close inverse relationship between the caries incidence of man and the fluorine content of the water supply has been firmly established by epidemiological studies in the United States (15 17) Great Britain (108) South Africa (74) and India (95) In one of the most important of these intensity of dental caries attack in 12–14 year old school children in eight suburban Chicago communities was inversely correlated with 0 0 to 1 8 p p m F in the drinking water (17) Dean and co workers (15) obtained confirmatory results in a later investigation involving 4 425 children aged 12–14 years living in thirteen cities of four states of the United States A graph summarizing the results of these studies with school children as presented by McClure (58) is given in Fig 23

From the results of Dean s epidemiological studies cited above Hodge (40) obtained linear relationships between the number of DMF (decayed missing and filled) teeth or the degree of mottling per 100 children from 12 to 14 years of age and the logarithm of the fluoride content of the drinking water The two lines intersect at about 1 p p m fluoride in the water indicating that this concentration may be described as "the point of maximum health with maximum safety This concentration must be subject to variation depending upon environmental temperatures which affect the volume of water consumed and upon the fluorine content of the rest of the diet

The major beneficial effects occur when the fluorine is ingested during the formative period of the teeth A 60–65% reduction in dental caries experience and about a 75% decrease in first permanent molar loss has been observed to be associated with fluorine ingestion at the recommended level throughout this early period (7 16) Deatherage (19)

reported reduced caries in adult male inductees exposed to fluoride waters starting even after their eighth birthday, but the benefits conferred by fluorine appear to be much less once the teeth have erupted. Much further critical work on this problem must be undertaken before the question of the benefits accruing to adolescents and adults from

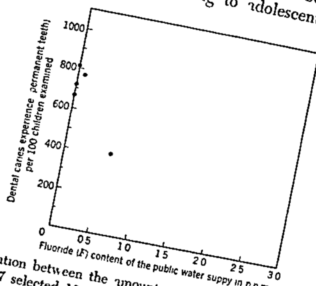


FIG. 23. Relation between the amount of dental caries (permanent teeth) observed in 7,257 selected 12 to 14 year old white schoolchildren of 21 cities of 4 states of the United States and the fluoride (F) content of public water supply. (Summary graph as presented by McClure (58) from the epidemiologic fluoride studies of Dean and co-workers 15, 17, 18)

fluorided waters can be answered. There is no doubt, however, that caries resistance acquired during tooth formation continues throughout adult life whether the consumption of fluorided water is continued or not (18, 67, 90). For instance, in a relatively recent report on the dental status of native inhabitants aged 20–44 years of two cities, one of which had a water supply virtually fluoride free and the other a supply containing 2.6 p p m F, the rates for decayed, missing, and filled teeth were 60% lower in the latter than in the former city *in each age group*. Further caries inhibition continued at an undiminished rate to the age of 44 years in the city with the fluorided water (90).

Experimental results give consistent support to the human caries-fluorine relation. These have been summarized by McClure (58) as follows:

1. Induced experimental caries in small animals is fluoride inhibited.
2. Dental tissues contain fluorine which may be classified as primary fluorine acquired during growth and calcification of the teeth; secondary fluorine acquired after tooth eruption and adsorbed; secondary enamel fluorine acquired possibly by local oral enamel surface adsorption.

3 Reduced solubility and surface changes in enamel and dentin are attributed to fluoride reactions on dental tissues *in vitro*
4 Fluoride may affect oral bacterial activity and exert antienzymatic effects possibly involved in causation of dental caries
5 The ameloblasts (enamel forming cells) are extremely sensitive to fluorine

b *The Mechanism of the Anti Caries Action of Fluorine* In spite of the very considerable volume of research undertaken a completely satis factory explanation of the anti caries action of fluorine has not yet been produced The possibility that the presence of fluorides in the oral cavity alters bacterial metabolism and inhibits enzyme activity is super ficially attractive Fluorine is a powerful antienzymatic agent and iodoacetic acid also an active antienzymatic substance inhibits caries when added to food (63) Further injected fluoride which does not enter the oral cavity is not caries inhibitory (6) On the other hand the effect of fluorine on salivary amylase is slight (59) and fluorine in saliva is not correlated with ingestion of fluorine (60)

Attempts to demonstrate differences in the fluorine content of sound and carious teeth have not been successful An early report by Armstrong and Brekhus (4) supported the view that the enamel (but not the dentine) of carious teeth contains less fluorine than that of sound teeth but this has not been confirmed by later work at least as applied to in dividual teeth McClure (61) analyzed the enamel and dentin of several hundred sound and carious teeth showing no evidence of fluorosis from nearly 100 individuals Significant differences in fluorine content were not revealed as is shown by the following figures

Sound teeth	Enamel 0 0102 ± 0 004% F	Dentin 0 0241 ± 0 001% F
Carious teeth	Enamel 0 0098 ± 0 003% F	Dentin 0 0225 ± 0 0007% F

The possibility remains of an increase of fluorine in the entire dentition which might account for an over all reduction in dental caries experience but individual carious teeth in a dentition clearly do not consistently carry less fluorine in the dentin or enamel than do sound teeth More over the best analytical procedures have so far failed to reveal an in crease in the fluorine content of teeth treated topically with fluoride (5) although most of the studies of topical applications of fluoride to tooth surfaces have demonstrated remarkable caries preventive effects (43)

The most likely explanation seems to be that fluorine influences the physical and biochemical properties of teeth and particularly of enamel surfaces *in vivo* perhaps by the surface adsorption of minute amounts of fluorine (too small to be detected with existing techniques) by the hydroxyapatite of the enamel with the production of a protective layer of acid resisting fluorapatite This hypothesis finds some support from

studies with radioactive fluorine which have shown that enamel, dentin bone and hydroxyapatite absorb fluorine according to the Freundlich adsorption isotherm (106) and from the finding that fluorine in concentrations as low as 1 p p m in the drinking fluids is effective in reducing the erosion produced *in vivo* on rats molar teeth by citrate and lactate drinking fluids (87) This latter finding suggests the occurrence of physicochemical changes which confer acid resisting properties upon the teeth

c *Dental Caries and Fluoride Medication* The establishment of the relationship between fluorine and human caries stimulated public health authorities especially in the United States to fluorinate communal water supplies deliberately in an attempt to duplicate mass population exposure to natural fluoride waters It has been reported that more than 200 city communities and between 3 500 000 and 4 000 000 people in the United States were being supplied with artificially fluorided water by the middle of 1952 (69) These important developments include several experiments in which one community is maintained as a fluoride free control Complete results from these investigations will not be forthcoming for several years but the impressive ten year Newburg Kingston (New York) study on water fluoridation has given extremely encouraging results after five years of operation (7 91) In this experiment the effect of adding sodium fluoride to the water supply of one city (Newburg), to maintain a level of 1 0–1 2 p p m F is compared with the neighboring control city (Kingston) which retains its fluoride free water supply Sodium fluoride is the most common fluoriding agent although sodium fluosilicate is being used in numerous cities including Washington D C (69)

Fluoridation of communal water supplies is not without its difficulties and its opponents especially in view of the narrow margin between the beneficial and toxic levels of this element After a careful review of all available evidence up to 1951 an expert Committee of the U S National Research Council made a lengthy report (71) in which the following statement sets out the position extremely well

the Committee recommends that any community which includes a child population of sufficient size and which obtains its water supply from sources which are free from or are extremely low in fluorides should consider the practicability and economic feasibility of adjusting the concentration to optimal levels This adjustment should be in accord with climatic factors and constant chemical control should be maintained With proper precautions this procedure appears to be harmless However it should be conducted under expert dental and engineering

supervision by the State board of health It should not be undertaken unless this can be provided How much reduction in the prevalence of caries will actually be realized in a particular community will vary according to local conditions The procedure will supplement but not supplant other dental health measures "

The success of water fluoridation led to numerous attempts to achieve the same results by the administration of fluoride containing tablets (101) There seems no obvious reason why dietary supplements of fluorine should not be just as effective in caries control as fluorided water Great care should however be exercised in their use and a really satisfactory demonstration of the value of fluoride tablets has yet to be made In connection with dietary sources of fluorine it is extremely interesting to note that tea in the quantities normally consumed by adults in some communities can supply just as much fluorine daily as would be supplied by the consumption of water, in ordinary quantities containing 1 p p m F In such communities however the children up to eight years old whose teeth are susceptible to fluorine drink very little tea Under these conditions no effect on caries incidence in the population from tea drinking would be expected Beneficial results have in general attended the topical applications of sodium fluoride and other fluorides (43) but as McClure has stated while the use of fluoride topically seems extremely promising variations in the procedure in the solutions used etc need to be studied"

Deliberate fluoridation of water supplies suggests the possibility of a cumulative toxic fluorosis At the moderate levels associated with caries prevention where adequate control is maintained the toxic hazard appears to be negligible Most of the fluorine balance studies with human subjects described in the previous section indicate that the amounts of fluorine retained by the body from dietary intakes up to 4-5 mg are exceedingly small It was found further in an extensive survey of the fluorine concentration of urine specimens of high school boys and of young selectees of the armed forces of the United States that upward of 90% of water borne fluoride (in concentrations ranging from 0 5 to 4 5 p p m F) is eliminated in the daily urine of teenage boys and young men (64) No effect upon the age height weight relations and bone fracture histories of these individuals from the fluoride waters was apparent (62) Further population studies are necessary for a completely convincing answer to this question but it seems highly unlikely that the continued ingestion of domestic waters containing 1 0-1 5 p p m F presents any public health problem

4 *Chronic Endemic Dental Fluorosis, or Mottled Enamel*

Mottled enamel was first described in the United States as occurring in humans at Colorado Springs in 1910 (26) In 1916 McKay (66) demonstrated that this defect of human dentition was a water borne disease and postulated that it was caused by the presence of some rare element in the drinking water during the period of calcification of the teeth It was not until 1931 that Smith *et al*, in Arizona (97), Churchill in Pennsylvania (11) and Velu in Morocco (103), independently established that mottled enamel was caused by the continual ingestion of toxic amounts of fluorine from the water Chronic endemic dental fluorosis in man due to the consumption of naturally fluorided waters has since been reported from every continent and most of the countries of the world including a restricted area in Queensland Australia (12)

a *Characteristic Features of Dental Fluorosis* The outstanding feature of dental fluorosis in man is the selective action of fluorine on the enamel of the permanent teeth Mottling of the teeth is characterized by chalky white patches distributed irregularly over the surface of the tooth These chalky areas are usually well defined and can readily be differentiated from the rest of the tooth Enamel which is mottled lacks the translucent glassy luster of normal enamel so that the mottled patches have a dead white turbid appearance Sometimes the whole tooth is dull white and opaque in appearance

The enamel is structurally weak and in severe cases there is a marked loss of enamel accompanied by pronounced pitting which gives the tooth surface a corroded appearance In all but very mild cases there is a secondary infiltration of staining which may show considerable variations in color ranging from dark brown through orange to yellow The stain is generally most pronounced in the upper lateral and central incisors Mild moderately severe and severe mottling of the teeth of individuals exposed to naturally fluorided waters in Arizona taken from the publication of Smith and Lantz (96) are shown in Fig 24

Mottled enamel is a defect mainly of the permanent teeth and develops only during their period of formation The enamel of adult teeth is unaffected by fluorine although the composition of the dentin and of the bones generally is changed by high intakes of fluorine The deciduous teeth may also be affected where the intakes of fluorine are unusually high At intakes common in endemic fluorosis areas the deciduous teeth remain unaffected Protection is afforded these teeth by the barriers imposed to transmission of fluorine from the mother by the placenta and by the mammary gland Pronounced mottling of the deciduous teeth has been observed however in some instances in the

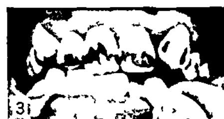
severely fluorotic areas of North Western Cape Province in South Africa (74) and of the Madras Presidency in India (94)

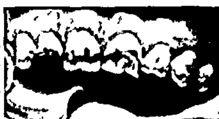
Changes in the size or shape of the affected teeth are of rare occurrence in mottled enamel areas The only oral abnormality commonly en













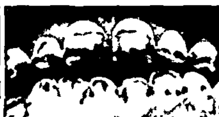


Fig 24 Types of mottled enamel found among the native born inhabitance of certain sections of Arizona 1 No mottling 2 Mild mottling 3 Moderately severe mottling 4 Severe mottling (Smith and Lantz 96)

countered other than those described is a tendency for the teeth to erupt somewhat later than in unaffected areas (A similar delay in the eruption of the incisors has been reported (38 76) in fluorotic sheep) A tendency towards gingivitis is also apparent in severely affected cases

In its mild forms mottled enamel has little public health significance

apart from esthetic considerations In the more severe forms, involving enamel hypoplasia and pitting of the teeth the unsightly appearance of the mouth may be accentuated by excessive wear on the teeth and mastication can be affected Mottled enamel is not usually accompanied by any other defects affecting the growth, health or well being of the individual Calcium and phosphorus metabolism are not impaired and there are no disturbances seriously affecting the skeletal bones or joints except in certain severely affected areas discussed later

b *Levels of Fluorine Associated with Mottled Enamel* It was stated earlier that 1 p p m fluorine in the drinking water can be described as "the point of maximum health with maximum safety The term eufluorosis has been proposed by P Adler *et al* (2) to express the optimal protection against dental caries without production of mottling Levels of intake equivalent to about 1 p p m fluorine in the water may be taken as the eufluorotic level and intakes above this level as being necessary for the production of significant mottling or other evidences of fluorosis It must be emphasized however, that levels in excess of 1 p p m fluorine in the water must continue for long periods of time for evidence of fluorosis to develop and that considerable individual variation in susceptibility exists Thus Dean (14) observed that different individuals of even a homogeneous group are diversely affected by the same concentrations of fluorine in the drinking water Certain children were found to have no mottled teeth, whereas others in the same family and using the same water were definitely affected In one area 289 children all using the same water were examined by Dean with the following results normal 9 questionable, 19 very mild mottling 44 mild 81 moderate, 98 moderately severe 32 and severe 6 It is apparent that this conforms to a normal monomodal frequency distribution and is merely a manifestation of normal biological variation

Classification of waters of differing fluoride content in terms of their capacity to induce mottling is clearly difficult because their effects will be influenced by the amounts of water consumed and by the level of fluorine intake from the rest of the diet The marked influence of the consumption of tea and of sea foods upon fluorine intake and assimilation has already been pointed out Evidence from studies in many areas suggests nevertheless that continuous ingestion during the period of dental susceptibility of water containing 1–2 p p m F will result in mild mottled enamel in 40–50% of children at 2–4 p p m F the mottling is accompanied by staining and some pitting and erosion at 4–6 p p m 80% of children are affected with deeply stained mottled pitted

and eroded teeth At over 6 p p m according to Ockerse, 100% of children are badly affected (73)

c *The Removal of Fluorides from Water* The formation of mottled enamel can readily be prevented but there is no known cure once defective enamel has been formed It is possible to bleach the disfiguring stain temporarily with oxidizing agents but the primary defect in the enamel is permanent Prevention has been accomplished in some localities by simply changing the water supplies but this is not always practicable Various methods of treating the affected waters by physical or chemical means to effect removal of the toxic fluorides have therefore been tried Even boiling for a few minutes is reasonably effective in reducing the fluorine content of fluorided water provided that the water is not left in contact with the precipitate formed (100) Such treatment is clearly impossible on a large scale

A variety of chemical compounds including aluminum sulfate activated alumina activated magnesium oxide and activated carbon has been used in filters (see van der Merwe 102) but they are neither reliable nor cheap The great affinity of fluorine towards the tertiary phosphates has been exploited with more success and at less cost H Adler *et al* (1) found that a tricalcium phosphate which was essentially a mixture of hydroxy apatite and tricalcium phosphate was very satisfactory for the removal of fluoride from water when used in conjunction with a filter bed Van der Merwe (102) advocates the use of an active defluorinizing agent derived from commercial superphosphate and draws attention to the possibilities of the much cheaper naturally occurring wavelite—an amorphous phosphate of iron and aluminum

d. *Fluorotic Osteosclerosis and Industrial Fluorosis* Generalized skeletal sclerosis is not generally associated with the consumption of fluorides in the drinking water In fact only one isolated case has been reported in the United States (48a) A suggestion that residence for 30–40 years in a district supplied with water containing 3–4 p p m F may lead to disturbances in ossification and to early spondylosis deformans has also come from England (41) Osteosclerotic symptoms have been reported however in severely affected endemic fluorosis areas in South Africa (72) and in India (94) although it is not certain that water borne fluoride is the sole factor involved

The unique character of the endemic fluorosis in the Madras Presidency is worthy of further mention Shortt *et al* (94) report that this condition is prevalent among natives who have been subjected for periods of 40 years or more to the influence of drinking water containing comparatively large quantities of fluorine In the initial stages mottling

of the permanent teeth (and sometimes the deciduous teeth) occurs in the children in the expected way with no other ill effects At about 30 years of age the first symptoms of more generalized poisoning set in Pain and stiffness of the spine and joints develop until the whole spine appears to be one continuous column of bone (the condition of poker back) and the ribs are rigidly fixed at their junction with the spine Breathing becomes entirely abdominal and in the advanced stages the victims exhibit a definite cachexia loss of appetite and emaciation followed usually by death Radiological examinations reveal excessive calcification of tendons and ligaments the presence of osteophytic out growths from various bones and almost complete synostosis of various joints

How far this condition is due to factors other than the excessively high fluoride waters is not yet clear but the clinical and radiological findings are very similar to the severe forms of skeletal fluorosis which have been reported among workers in factories using or producing fluorine com pounds A very complete picture of industrial skeletal sclerosis is given in the monograph by Roholm (89)

Fluorine and Enzyme Action

Fluorides exhibit a remarkable reactivity towards certain enzymatic processes in some instances causing a marked stimulation and in other almost complete inhibition A comprehensive review of this subject up to 1945 has been prepared by Borei (10) Those enzymes which require as catalysts metals such as calcium magnesium manganese iron copper and zinc, are particularly affected owing to formation of fluorine metal complexes Fluorine may also combine with the prosthetic groups of phosphoproteins thus inhibiting activity by the formation of fluoro phosphoprotein complexes Enolase for instance, has been shown to be inactivated by the formation of magnesium fluorophosphate (107)

The possibilities of specific or general disturbances in enzymatic proc esses in the tissues of animals suffering from chronic fluorine poison ing have been very little studied The similarity of the symptoms of chronic fluorosis in guinea pigs to the scorbutic syndrome in this species suggested an interference with ascorbic acid or its function in the fluorotic animal This suggestion was not completely confirmed but definite indications were obtained that ascorbic acid deficiency and the deleterious effect of chronic fluorine toxicosis result primarily from disturbances in specific phases of cellular respiration (82)

An effect on bone phosphatase is probably the explanation of the interesting finding that extremely low concentrations of fluoride have

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CHAPTER 11

SELENIUM

Interest in selenium as a trace element is almost entirely confined to its toxic properties in animals. Conclusive evidence of an essential function for this element in plants, animals, or microorganisms has not yet been produced, although field observations suggest that it may be essential for certain plant species known as "indicator plants." It has been reported that selenium is a stimulating, if not an essential element for these species (57).

Selenium was shown to be toxic to animals as early as 1842 (21) and the ability of plants to absorb selenium from soils to which it had been added was first demonstrated in 1880 (5), but not until 1933 (47) and 1934 (3), when the naturally occurring diseases of livestock known as "alkali disease" and "blind staggers" were shown to be manifestations of chronic and of acute selenium poisoning, respectively, did this element attract much biological attention. An understanding of these serious field problems was vital to the continued settlement and development of large areas in the semi-arid Great Plains of North America where selenium poisoning in livestock was enzootic. In consequence, intensive studies of the distribution of selenium in rocks, soils, plants and animal tissues were undertaken, the absorption, excretion, toxic properties and mode of action of this element and its compounds in animal species, including man, were investigated, and prophylactic measures were sought. The results of these studies are outlined in the following sections.

I. "Alkali Disease" and "Blind Staggers" of Livestock

1. *Historical*

The first authentic written record of selenium poisoning in livestock appears to be that of Madison (see Moxon 31), who reported in 1856, the occurrence of a fatal disease in cavalry horses consuming native vegetation at Fort Randall in Nebraska. This disease was characterized by loss of hair from mane and tail and soreness of the feet. Soon after settlement of this part of the North American continent towards the end of the nineteenth century, losses of grazing stock and of stock fed grains and roughages grown in the area were experienced from an unknown malady with symptoms similar to those observed in Madison's horses. Early settlers gave the name "alkali disease" to this condition

because of their belief that it was caused by the "alkali" (high salt) waters and seepages of the area. The name has persisted although the theory behind it has long been disproved. At about the same time that the first reports of alkali disease in farm stock appeared, serious losses of grazing stock from another and equally baffling disease were reported from parts of Wyoming, to which the more descriptive name of "blind staggers" was given. For many years these were regarded as unrelated maladies and were investigated independently in Wyoming and South Dakota.

Intermittent investigation of the two diseases was undertaken almost from the beginning of the century (31). Suspected causal agents such as "alkali" waters, ergot, various molds, and poison plants were eliminated, and the enzootic and noninfectious nature of the diseases was established. From 1929, when K. W. Franke began active work on the alkali disease problem at South Dakota, substantial positive progress began to be made. Franke carefully described the symptoms of poisoning in different farm animals, tested feeds from affected areas on laboratory animals and showed them to be toxic, and deduced from the nature of the symptoms the possibility that the disease was caused by the presence in these feeds of arsenic, thallium, or other less common toxic elements (31). Specialists of the U.S. Department of Agriculture were then called into consultation, and a systematic search for trace elements in grain which Franke had found to be toxic by bioassay led to Robinson's discovery of the presence of selenium (47).

Subsequently all the cereal grains shown to be toxic by Franke were found to contain selenium, and high concentrations of this element relative to those of disease-free areas were detected in the soils and plants of the known affected regions. The possibility that other toxic elements were also involved, such as tellurium, molybdenum, and arsenic, which were found to be present in some seleniferous soils and plants (3), was not dispelled until Franke and Potter (13) demonstrated that the addition of sodium selenite or selenate to an otherwise normal diet induced symptoms in the rat which appeared identical with those produced by the natural toxicant. Further, Franke and Painter (12) removed nearly all of the selenium from a hydrolysate of a seleniferous protein and found that the hydrolysate so treated was no longer toxic.

During this time Beath and co-workers in Wyoming were making detailed studies of the chemical and physiological properties of several native species of the genus *Astragalus*, which they suspected to be associated in some way with the occurrence of blind staggers. In 1932 Taboury in France published his researches on the occurrence of seleni-

um in certain vegetation among which was a species of Sium (55). Since *Sium cicutaefolium* was known to occur in blind staggers areas this species was examined and found to be rich in selenium (3). This was the beginning of a series of experiments demonstrating the relationship of this disease to the ingestion by stock of particular native species carrying exceptionally high selenium concentrations and the establishment of blind staggers as an expression of acute selenium poisoning.

Seleniferous soils and vegetation were found to occur over considerable areas of the Great Plains including parts of Canada but similar conditions have not been reported from outside the North American continent except for a small severely affected area in Ireland (57a). In North America the selenium is believed to be of volcanic origin and to be largely, but not entirely, confined to heavy clay ("gumbo") soils (34). The toxicity of vegetation growing upon such soils has been shown to be dependent upon a variety of factors such as the plant species and the chemical forms in which the selenium is present in the soil, as well as upon the total concentrations of selenium which the soils contain. The significance of these factors to the incidence of selenium poisoning is brought out below.

2. *Symptoms of Selenium Poisoning*

Selenium poisoning as it occurs naturally has been divided historically into two types—the chronic type, alkali disease, and the acute type, blind staggers. All degrees of poisoning between these two extremes exist and are experimentally demonstrable. The symptoms therefore vary with the level of selenium ingestion and with the length of time over which the ingestion takes place. Appreciable species differences also exist. The general symptoms of chronic selenium poisoning are nevertheless as follows: dullness and lack of vitality; emaciation and roughness of coat; anemia; stiffness and lameness due to erosion of the joints of the long bones; soreness and sloughing of the hoofs; loss of hair from the tail and from the mane of horses and body of pigs; atrophy of the heart ("dish rag" heart), and atrophy and cirrhosis of the liver. Death often results from starvation and thirst because, in addition to loss of appetite, the lameness and pain from the condition of the hoofs are so severe that the animals are unwilling to move about to secure food and water. Acute selenium poisoning is characterized in the final stages by blindness, various degrees of paralysis, abdominal pain, salivation and grating of the teeth. Death usually results from failure of respiration.

A high incidence of anemia is found in both alkali disease and blind staggers. In fact Moxon and Rhian (37) assert that the hemoglobin

is affected by concentrations of selenium too low to cause manifest symptoms of poisoning in other farm animals. The eggs are fertile but a varying proportion produce grossly deformed embryos (14) (Fig. 27). The chicks which do hatch are generally weak and exhibit an abnormal wiry condition of the down. Monstrosities similar to those found in

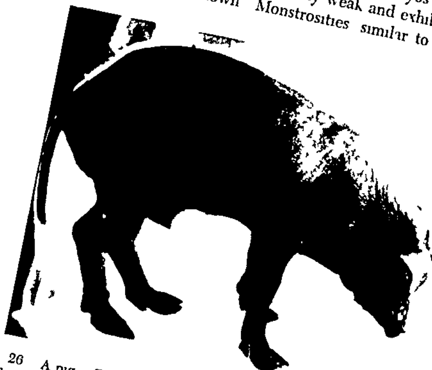


FIG. 26. A pig suffering from alkali disease (chronic selenium poisoning). Note the diseased feet, thin hair and poor condition. (Moxon 31)

chick embryos from eggs laid by hens fed toxic grains were observed by Franke *et al.* (9) following the injection of selenium salts into hens' eggs quite early in the alkali disease investigations.

3. *Minimum Toxic Levels of Ingested Selenium*

The minimum level of selenium intake in the food that will lead to symptoms of chronic selenium poisoning cannot be given with any precision for any species. There are considerable individual variations, some species differences, and a marked effect of other dietary factors, notably the protein content of the ration. The influence of protein is considered later. The form in which the selenium is administered also has an important bearing on the minimum toxic level. Elemental selenium, owing to its insolubility, is relatively nontoxic, whereas the readily soluble selenates and selenites have a toxicity comparable per unit of selenium with the selenium in toxic grains and roughages.

The lower limit of selenium toxicity to animals that is the minimum level at which this element will accumulate in the tissues and produce the characteristic signs of selenium poisoning is placed by Munsell *et al* (40) at 3–4 p p m Se Such levels of selenium in rations based on seleniferous grains have no deleterious effects on the growth of chicks or



Fig 27 Deformed chick suffering from selenium poisoning (Photo kindly supplied by Dr O E Olson)

on the hatchability of eggs but at more than 2 5 p p m the meat and eggs contain concentrations of selenium in excess of the suggested tolerance limit for foods (35 40) At a level of 5 p p m hatchability is reduced slightly and at 10 p p m it is reduced to zero (14) Symptoms of chronic poisoning in rats and dogs are common when the rations contain 5–15 p p m Se and subacute symptoms are produced when the level is 15–25 p p m or more Almost complete refusal of food and death in a short time was observed (37) in dogs given rations to which 20 p p m Se as sodium selenite was added Young pigs fed seleniferous corn containing 10–15 p p m Se develop typical symptoms of poisoning within 2–3 weeks (37) Presumably a longer period of feeding of

rations containing somewhat lower levels of selenium than these would result in the development of the same symptoms

The actual intakes of selenium by stock grazing seleniferous areas are difficult to determine, because of the great variability in the concentration of this element in different plant species and the impossibility of knowing the extent to which particular plants are actually consumed Edible herbage species generally contain 10–20 p p m Se or less when growing on seleniferous soils Such concentrations produce typical symptoms of alkali disease in cattle when ingested for a period of several weeks or in some cases several months On the other hand, the consumption of converter plants such as *Astragalus* species, which contain several hundred or even several thousand parts per million of selenium (Table 43), will result in acute selenium poisoning (blind staggers) within a few days

4 *Mode of Action of Selenium*

Much remains to be learned of the nature of the basic metabolic disturbances which occur in chronic and acute selenium poisoning The element is known to be readily absorbed from seleniferous diets and to accumulate in the body tissues and fluids apparently in combination with protein but the exact ways in which such selenium interferes with tissue structure and function are not yet fully understood

Selenium has been known for years to have a direct effect on certain unicellular organisms and enzyme systems (26) and to inhibit alcoholic fermentation by yeast (6 45) An injurious effect of selenium compounds on certain of the enzymes concerned with cellular respiration has also been demonstrated (7) These studies disclosed an inhibition of oxygen consumption by tissues *in vitro* apparently through the poisoning of succinic dehydrogenase More recently it has been shown that the liver succinic dehydrogenase levels of rats fed seleniferous diets are reduced below normal (24) Whether this effect is sufficient to account for the various manifestations of selenium poisoning remains to be determined No other enzymes have yet been found to be affected in the living tissues of selenized animals but the *in vitro* studies of Wright (60) with various tissues to which sodium selenite or selenate has been added in concentrations of the order prevailing in the intact animal receiving a minimum lethal dose of these substances indicate a general poisoning of the dehydrogenating enzymes and no impairment of the cytochrome indophenol oxidase system An inhibiting effect of selenium has been observed on urease but not on cholinesterase catalase or liver arginase (61) These findings together with the partial protective action

of reduced glutathione suggest strongly that the injurious effects of selenium are due to the removal of sulfhydryl groups essential to oxidative processes

Little is yet known of the nature of the selenium compounds formed in the tissues of selenized animals although it is possible to argue by analogy with the position in plants that this element reacts with the sulfur containing amino acids and forms compounds with them in which the sulfur is partially replaced by selenium A crystalline, amino acid complex containing selenium and sulfur has been isolated from highly seleniferous *Astragalus pectinatus* (18) and there is evidence that in toxic cereal grains the selenium occurs in the protein fraction replacing some of the sulfur in cystine and perhaps methionine (33) These findings suggest that selenium analogues of the sulfur containing amino acids of the tissue proteins may exist in selenized animals Crude glutathione containing some selenium has in fact been isolated from the blood of selenized cattle (2)

5 *Diagnosis of Alkali Disease*

The symptoms of chronic selenium poisoning are generally easily recognized so that no problem of diagnosis normally arises There are some cases however in which the clinical signs are insufficient to establish this condition unequivocally and a simple laboratory diagnostic test has long been sought The analysis of feeds for selenium content although not simple, is adequate for this purpose where the animals are hand fed and the actual consumption is known Under grazing conditions feed analyses are relatively unsatisfactory because of the very great variability in the selenium content of different plants (see Section II of this chapter) and the difficulty of determining what plants have been eaten and in what quantity Since selenium accumulates in the tissues of animals consuming seleniferous feeds the analysis of some part or parts of the animal appeared to be the most likely solution to the problem It is of course necessary to obtain such tissues without sacrificing the animal

The blood and the hair especially the latter are the most readily accessible tissues from the living animal These have been subjected to systematic study from cattle on seleniferous range and on ranges where alkali disease had never been observed (41) Determination of selenium on either blood or hair was found to be a valuable diagnostic aid provided that sufficient animals were sampled to allow for the large individual variability and that the marked seasonal changes due to the shedding of old hair and the growing of new were recognized

The testing of hair has proved of more practical value than the testing of blood because of the ease with which hair samples can be collected in the field by clipping and can be despatched without deterioration to the laboratory.

Samples of hair from cattle from nonseleniferous areas, that is, areas where alkali disease had not been reported, gave values ranging from under 1 p.p.m. to slightly over 4 p.p.m. Se (41). All of these samples were collected during the fall when the highest values would be expected. From these results Olson *et al.* (41) conclude that, except during the late winter and early spring months, an average selenium content of less than 5 p.p.m. (in the hair) indicates alkali disease symptoms should not occur in a herd. By contrast, from samples taken several times throughout one year, the average selenium content of the hair of 28 yearling cattle on a ranch where selenium poisoning had long been a problem was well above this figure. Individual values varied over a fairly wide range and there was a definite seasonal variation with an increase in selenium content throughout the growing season followed by a decline which probably reflected shedding of old hair. Excluding the first samplings, the selenium content of the hair of these animals averaged over 10 p.p.m. The average values obtained in this study extending over 17 months are presented in Fig. 28. Olson and co-workers suggest from data as yet unpublished that the values are applicable to pigs as well as to cattle.

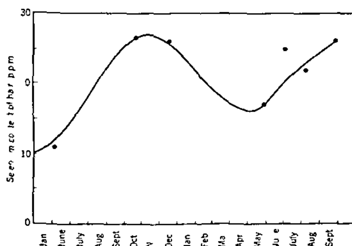


Fig. 28. The average selenium content of the hair of cattle grazing a seleniferous range. (Graph kindly supplied by Dr. O. E. Olson.)

6 *Prevention and Treatment of Alkali Disease*

Since alkali disease and blind staggers result from the ingestion of forages and grains containing toxic amounts of selenium and these in turn result from the growth of the plants on soils abnormally high in available selenium, these diseases could be overcome and the seleniferous areas rendered safe for stock by treatments that (a) limit the absorption of selenium by plants so that the concentrations in the whole plant or seed do not reach injurious levels or (b) reduce the absorption or promote the excretion of selenium by animals so that injurious amounts do not reach or are not retained in the tissues or (c) inhibit or antagonize the toxic effects of selenium within the body tissues or fluids

Only treatments involving the last of these alternatives have so far been found at all successful although hopes were raised that sulfur treatment of soils would limit selenium absorption by plants and mention should be made in relation to the second alternative form of treatment of the interesting work of Moxon on the promotion of selenium excretion in animals The administration of bromobenzene to rats and dogs fed a seleniferous ration or of bromobenzene benzene or naphthalene to steers grazing on seleniferous range was found to increase the urinary selenium output (39)

a *Effect of Sulfur Additions to Seleniferous Soils* The addition of sulfur to cultures and soils to which sodium selenate had been added was shown by Hurd Karrer (20) to inhibit the absorption of the added selenium by plants As a result of this finding the application of sulfur to soils was advocated as a means of preventing the production of toxic vegetation Unfortunately studies of the effects of added sulfur to natural seleniferous soils showed conclusively that such treatment was ineffective under the conditions which exist in the affected areas Franke and Painter (11) added sulfur as ground sulfur and as gypsum to soils in a toxic area and failed to secure any inhibition of selenium absorption by corn or by wheat plants This is not unexpected in view of the fact that such soils are generally already saturated with sulfur in the form of gypsum Moreover a large proportion of the available selenium in these soils is frequently in organic combination Organic compounds of selenium are unlikely to be greatly affected by changes in the inorganic sulfur selenium ratio

b *The Effect of Dietary Factors on Selenium Poisoning* Early in the investigation of alkali disease attempts were made to find a dietary factor or factors which would prevent or reduce the toxicity of selenif

erous diets (31) Various feeding trials with rats which included mineral and vitamin supplements gave negative results but selenium poisoning was found to be less severe on high protein rations than on those with low or normal protein contents This finding was later substantiated and extended by Smith and others (27 50 52) Thus 10 p p m of wheat selenium fed in a diet containing 10% protein was found to be highly toxic to rats whereas the same amount of selenium fed in an isocaloric diet containing an additional 20% protein as casein scarcely produced any toxic effects (50)

The protective effect of high protein intakes is not confined to casein although there is some disagreement among different workers on the potency of various protein sources For example Smith (52) has shown that wheat protein lactalbumin ovalbumin zein yeast protein desiccated liver, and even gelatin afford protection against the toxic effects of seleniferous wheat on rats Moxon (37) on the other hand found crude casein and linseed meal to be the only commercial proteins to prevent the characteristic liver lesions in rats while dried liver purified casein and whole milk powder gave good growth but failed to prevent or alleviate pathological lesions of selenium poisoning and meat scraps tankage and corn gluten meal neither induced good growth nor prevented the lesions of selenium poisoning Further in experiments with dogs it was observed that linseed meal and dried liver gave good growth and protection against the effects of selenium but crude casein tankage and corn gluten meal were completely ineffective (37)

These findings reveal species differences which invite caution in extending such treatments to farm animals without further experimentation especially as the mechanism of the protein selenium antagonism is unknown It seems likely that this antagonism is related in some way to the inhibiting effect of selenium on certain dehydrogenating enzymes mentioned previously and that the sulfur containing amino acids play some part in the protein effect Even in this latter respect however the position is not entirely clear Thus Smith and Stohlman (52) found neither cystine nor methionine as such to be of value in alleviating the toxicity of selenium and Klug *et al* (22) showed that dietary methionine at levels of 0 5–2 0% was ineffective in the protection of rats against seleniferous feeds whereas Schultz and Lewis (49) demonstrated a beneficial effect from methionine when added to a methionine deficient diet

c *Arsenic Treatment of Selenium Poisoning* In 1938 Moxon (32) reported the alleviation of selenium poisoning in rats by arsenite This surprising finding arose from experiments designed to test the effect of

various elements in the drinking water on the toxicity of a seleniferous diet At a concentration of 5 p p m fluorine, molybdenum, chromium vanadium cadmium, zinc cobalt, nickel and uranium were all found to cause increased mortality, lead had no effect and tungsten decreased mortality and prevented to a limited extent, the typical liver lesions but arsenic, at a level of 5 p p m in the drinking water completely prevented all symptoms of selenium poisoning (32, 37)

Arsenic has since been used successfully to counteract selenium poisoning in pigs dogs chicks and cattle (37, 38) Sodium arsenite and arsenate are equally potent but arsenic in the form of certain organic compounds is less effective and arsenic sulfides (AsS and AsS_3) are ineffective (37) Either 5 p p m or 10 p p m arsenic as sodium arsenite in the drinking water completely prevents any sign of selenium poisoning either on natural seleniferous diets or on diets made equally seleniferous by additions of sodium selenite or the selenium analogue of cystine (37) These levels of arsenic produce no signs of arsenic poisoning either when consumed with control diets or with seleniferous diets and they afford protection against much higher intakes of selenium than those which will without arsenic, induce some signs of selenium poisoning Nevertheless, arsenic is sufficiently toxic to animals to make its general use on farms and ranches somewhat impractical For this reason arsenic compounds of a lower order of toxicity namely arsanilic acid and 3 nitro 4 hydroxyphenylarsonic, have been tried on the rat (16) Both these compounds give partial protection against chronic selenium poisoning in rats but even at the highest levels which have been used (arsanilic acid 0 025% and 3 nitro 4 hydroxyphenylarsonic acid 0 009%) complete protection has not been observed

The arsenic selenium antagonism cannot be explained in terms of reduced selenium absorption or increased excretion, since the selenium content of the blood and tissues of arsenic treated animals is not significantly different from that of untreated animals showing signs of selenosis (23 46) It appears to be due at least in part, to the release of the inhibition of succinic dehydrogenase brought about by selenium (24) The liver succinic dehydrogenase levels of rats fed seleniferous diets are reduced, and can be restored to normal, after a short period of depression by the inclusion of arsenic in such diets Whether the action of arsenic in alleviating chronic selenium poisoning is due wholly to this effect on succinic dehydrogenase remains to be determined

II Selenium in Plant Materials used as Foods

The concentration of selenium in plants varies from amounts too small to be detected by available methods of analysis to levels as high as several thousand parts per million This wide variation results from the operation of a great many factors among which the plant species and the amount and chemical form of the selenium in the soil upon which the plant is growing are the most important

1 *The Influence of the Selenium in the Soil*

A general relationship exists between the total concentration of selenium in the soil and the selenium content of the plants growing thereon Selenium free soils naturally produce selenium free plants and soils high in selenium tend to produce plants which are also high in this element But soils of relatively high selenium content are known which do not produce plants high enough in selenium to be injurious to animals (25) Analysis of soils for total selenium is therefore not an entirely reliable index of the toxicity of the herbage Nevertheless soils containing more than 0 5 p p m selenium are regarded by Moxon (31) as potentially dangerous

Of far greater significance than total selenium in determining the concentration in the plant is the chemical form or forms in which the selenium occurs in the soil Different selenium compounds vary greatly in their availability to crop plants Seleniferous soils may contain soluble selenates which are readily absorbed by plants selenites which are moderately well absorbed or insoluble basic iron selenite which does not appear to be absorbed at all by ordinary crop plants although it is readily taken up by certain converter plants Organic selenium compounds also exist in seleniferous soils These appear to be well absorbed by plants (34 58) Since the proportions of these various chemical forms of selenium can vary markedly in different seleniferous soils and the availability of these compounds to plants is so different the concentration of selenium in any particular plant species will obviously not be highly correlated with total soil selenium The great variations which can occur between the selenium content of the plant and that of the soil upon which the plant was growing are illustrated in Table 43 Presumably these variations are primarily a reflection of differences in the types of selenium compounds present in the soils

2 *Species Differences*

The plant genus or species is a further factor of very great significance in determining the selenium concentration of vegetation growing

upon a particular soil Miller and Byers (30a) divide plants into three classes according to their capacity to assimilate selenium (1) those showing a limited tolerance for selenium and absorbing only small amounts (up to about 5 p p m) from seleniferous soils, most grasses and garden vegetables belong to this class (2) those which absorb moderate amounts (up to about 30 p p m) without injury to themselves all the cereals (and also onions) belong to this class and (3) those which absorb selenium readily and may accumulate up to several thousand p p m in their tissues These latter are mostly perennial plants of the orders *Leguminosae, Cruciferae,* and *Compositae,* and in particular some species of the genera *Astragalus Stanleya Oonopsis* and *Xylorrhiza* They include the important converter plants, whose special significance to the problem of selenium poisoning in the field is discussed below Included also are certain plant species, known as indicator" plants, which grow only where there is selenium in the soil Data showing these species differences taken from the publications of Franke and Moxon, of Byers and of Beath, as presented by Painter (42), are given in Table 43

The selenium contents of plant materials in the second and third of Miller and Byers classes are considered more fully in the separate sections which follow In relation to the first group of low selenium plants it may be mentioned that Moxon *et al* (34) found a number of grass species growing on seleniferous soils in South Dakota to average only 1–5 p p m Se An exception to the generalization that grasses take up comparatively little selenium from such soils was provided by Western wheat grass (*Agropyron smithii*) which contains the much higher average of 11 p p m Se Similarly, garden vegetables were found by Smith and Westfall (51) to be generally low in selenium These workers obtained the following values beans, 0 4–2 0 beets 0–1 2 cabbage 0–4 5 carrots, 0 4–1 3 peas 0 4–2 0 potatoes 0 2–0 9 and tomatoes, 0–1 2 p p m Se On the other hand onions growing under the same conditions ranged in selenium content from 0 to 17 8 p p m which brings them into the second group of plant species

3 *Selenium in Cereal Grains*

Special interest attaches to the selenium content of cereal grains because of their importance in the rations of pigs and poultry in seleniferous areas and their possible significance as a source of selenium in human dietaries Robinson (48) determined the selenium content of wheat from various parts of the world including nonseleniferous areas in the United States The samples ranged from 0.1 to 1 9 p p m but

the highest amount of selenium found in any sample grown outside the North American continent was 1 5 p p m The maximum selenium content of composite samples of Saskatchewan wheat was also found to be 1 5 p p m but one individual sample contained as high as 4 0 p p m (56) It is apparent that small concentrations of selenium with occasional values as high as those found in seleniferous areas are of widespread occurrence in this cereal A similar range of data does not appear to be available for other cereals but species differences are small judging by the evidence from seleniferous areas Thus the following figures have been reported (54) for the selenium content of cereal grains grown in seleniferous districts wheat 1 1–18 8 corn 1 0–14 9 barley 1 6–5 7 oats 2 0–10 0 and rye 0 9–3 8 p p m Values as high as 30 p p m have however been reported for samples of seleniferous wheat from South Dakota (37) and Hurd Karrer (19) produced wheat containing 220 p p m Se by adding sodium selenate to soil in the glasshouse

Most of the selenium in wheat corn and barley is associated with the protein (43) and is therefore well distributed throughout the grain Analysis of the mill fractions of four wheats of greatly different selenium contents revealed in all cases the highest concentrations in the bran which was also highest in nitrogen content and substantially higher concentrations in the shorts (pollard) than in the flour (Table 44) These findings are probably of considerable importance from the stand

TABLE 44
SELENIUM DISTRIBUTION[a] IN SELENIFEROUS WHEAT (36)

Sample number	Whole grain	Patent flour	All flour fractions	Shorts	Bran
1	4 8	4 08	4 1	5 5	5 9
2	5 8	4 02	4 05	6 3	8 7
3	23 3	19 09	19 06	24 8	33 4
4	63 0	53 26	53 58	77 2	88 4

[a] Measured in p p m on the dry basis

point of human nutrition in seleniferous areas since normally the bran and pollard fractions of wheat do not constitute more than a minute proportion of human dietaries

4 *Selenium in "Converter and Indicator Plants*

The great differences in ability to take up selenium from the soil that exist among different plant species assume special significance in the case of a few species belonging to genera which have already been enu

merated, known as 'converter' plants These species do not occur, or are very rare in nonseleniferous areas, for which reason they are sometimes called 'indicator' plants Such plants not only absorb and accumulate very large amounts of selenium but they are capable of doing so on soils that contain this element in insoluble or relatively insoluble forms These forms of selenium cannot be absorbed by crop plants and other species No satisfactory explanation of the special ability of converter plants to absorb large amounts of selenium is available

In certain of the converter plants a proportion of the selenium is volatile and therefore escapes into the atmosphere but most of it is returned to the soil in an organic form upon the death of the plants This organic selenium then becomes available for absorption by crop plants In other words converter plants convert selenium that is not generally available into selenium that is available to all types of plants They thus play an important dual role in the incidence of selenium poisoning in livestock in the field Firstly they constitute the type of herbage which produces blind staggers in stock because the concentrations of selenium that they carry are sufficiently high to induce this acute manifestation of selenosis in grazing animals Native stock usually avoid converter plants except in times of drought or overgrazing but stock brought in from other areas are not so discriminating and deaths frequently result Secondly, they contaminate the soil about them with selenium that can readily be absorbed by other plant species On some soils this may not be important because the selenium is already largely present in soluble forms and concentrations injurious to livestock may be present in most or all plant species On other seleniferous soils however, injurious levels of selenium only occur in all plants by virtue of the presence of converter plants It is apparent, therefore that converter plants can intensify the severity of selenium poisoning and extend its incidence

Selenium concentrations typical of converter plants growing on seleniferous soils are presented in Table 43 It will be noted that the levels range from under 100 p p m to over 9000 p p m but lie mostly between 1000 and 3000 p p m The facility with which these plants absorb insoluble selenium from the soil is illustrated by an experiment carried out by Beath *et al* (3) These workers added finely divided elemental selenium plus rotted horse manure, to a selenium free soil to give a level of 25 p p m Se, and grew wheat and 2 converter plants (*Astragalus bisulcatus* and *A pectinatus*) upon the soil so treated The *Astragali* species averaged no less than 1150 p p m Se after 3 months growth compared with 6 3 p p m in the wheat plants when they were 8 inches high The ability of converter plants to convert insoluble selenium into

a form capable of absorption by wheat plants was demonstrated by a further experiment. When grown on seleniferous shale young wheat plants were found to contain 2 p.p.m. Se, whereas similar plants grown on the same seleniferous shale to which finely ground converter plants had been added contained 227 p.p.m. Se.

III Selenium in Animal Tissues and Products

No selenium can be detected by available methods of analysis in the tissues or organs of animals fed normal selenium-free rations. The concentrations present in the tissues in selenium poisoning depend primarily on the amounts and forms of selenium administered and on the length of time of administration, but individual variability is extremely large—too large, in fact, to permit worthwhile conclusions as to species differences, if any such exist. The highest concentrations occur fairly consistently in all species in the liver and kidneys, with occasional high values in the blood, pancreas, spleen, and the hoofs of cattle, and generally much lower levels in other tissues. Average values for several species together with the range recorded are presented in Table 45. They illustrate the great individual variability mentioned earlier, as well as the general relationship between level and period of selenium intake and the selenium content of the tissues. At intakes that result in symptoms of chronic selenium poisoning the concentrations in the tissues continue to rise for periods of weeks, months, or even years, depending upon the dietary levels of selenium, but they tend to reach a maximum beyond which excretion appears to keep pace with absorption. Selenium is not therefore continuously cumulative in the tissues.

Under conditions of acute poisoning, at which point the intakes are very high, selenium reaches all the tissues except the hide and hair, with the highest levels in the kidney, liver, lungs, and spleen. Its occurrence in less active tissues appears to be directly related to the time of survival of the animal (30). In the rat given a single subtoxic injection of sodium selenite containing radioactive selenium, the greatest concentrations were found in the liver, muscle, gastrointestinal tract, and blood, with lesser amounts in the lungs and spleen and none at all (after 24 hours) in the skin, fur, teeth, or long bones (28).

Average values obtained for 8 steers grazing on seleniferous range for 2 seasons were 3.0 p.p.m. in the hoof, 2.2 p.p.m. in the kidney, and 1.2 p.p.m. in the liver, all on the fresh basis. All other parts of the carcass were lower in selenium content, including the muscle, which contained 0.68 p.p.m. (37). For 30 steers which had been on the same range for 3 seasons the average values were 5.7 p.p.m. in the liver, 4.1 p.p.m. in

TABLE 45
SELENIUM[a] IN ANIMAL TISSUES

Selenium content of diet (p p m)	Species	Period of feeding (weeks)	Liver	Kidneys	Heart	Spleen	Lungs	Intestines	Brain	Pancreas	Blood	Muscle	Bones	Hide and hair	Reference
20	Dogs (10)	Several	31.1 (12–67)	28.4 (6–67)	5.0 (tr–17)	4.95 (0–23)	3.50 (0–7)	2.20 (0–10)	2.00 (0–10)	7.40 (0–24)	0.28[b] (0–0.4)	2.53 (0–10)	0.86 (0–2.5)	—	(31)
10.3	Rats (15)	10–12	8.9 (6–12)	18.5 (12–28)	—	5.0	3.3	—	6.1	4.3	7.2	4.8 (2.3–6.5)	11.8	5.8	(23)
10.3	Rats (10)	8	9.5 (2.6–13)	18.8 (12–26)	6.1	5.8	4.5	—	3.8	5.2	6.2	7.5 (6.3–9.4)	5.5	4.8	(23)
1.25	Hens (4)	5	3.1 (1.0–5)	0.9 (0–2.6)	0.25 (0–1.0)	4.2 (0–16)	1.5 (0–4.6)	2.8[c] (0.2–6.5)	—	—	—	2.2 (white) 2.6 (dark)	—	—	(35)
2.50	Hens (4)	5	3.8 (0–8.0)	5.7 (3–10)	0.0	13.6 (1–30)	1.0 (0–4)	3.3 (1.2–6)	—	—	—	2.6 (white) 1.9 (dark)	—	—	(35)
5.0	Hens (4)	5	7.6 (4–14)	6.0 (3–8)	7.6 (0–23)	7.0 (4–12)	5.2 (2–11)	5.2 (0.4–12)	—	—	—	5.2 (white) 3.8 (dark)	—	—	(35)

[a] Measured in p p m on the dry basis [b] Red Cells only [c] Gizzard

the kidney and 3.0 p.p.m. in the muscle (37). Dudley (8) fed sodium selenite or plants containing selenium to sheep, calves, horses and pigs and found the selenium to occur mainly in the liver and kidneys (4–25 p.p.m.) of the acute cases and in the same tissues plus the spleen in the chronic cases. Selenium occurred in the blood in concentrations ranging from 7 to 27 p.p.m., mainly in the red corpuscles in a proteinlike selenium complex. In cats also the selenium in the blood has been shown to occur mainly in the erythrocytes (51).

The value of selenium estimations on blood and hair in the diagnosis of chronic selenium poisoning was discussed earlier. Hair gradually accumulates this element over long periods of ingestion at relatively low intakes, whereas high concentrations in the skin and hair, fur or feathers are not characteristic of acute selenium poisoning.

Moxon and Poley (35) fed hens seleniferous grain rations for a period of five weeks and determined the selenium content of the eggs produced and of the tissues and organs of the birds at the end of this period. The results obtained for certain parts of the carcasses are included in Table 45. They reveal marked individual variability but indicate a general increase in selenium concentration in the tissues as the intakes of dietary selenium rise from 1.25 to 5.0 p.p.m.

The selenium content of eggs also increases as the level of this element in the diet of the hen rises from 2.5 to 10.0 p.p.m. This is shown in Table 46, which demonstrates further that, unlike most trace elements, selenium is preferentially deposited in the albumin.

TABLE 46

SELENIUM CONTENT[a] OF HENS' EGGS (35)

Se content of ration	Whites	Yolks
2.5 p.p.m.	11.3	3.6
5.0 p.p.m.	19.0	5.9
10.0 p.p.m.	41.3	8.4

[a] Average p.p.m. Se on the dry basis.

It was mentioned previously that suckling foals, calves and pigs may develop typical signs of alkali disease, indicating that selenium readily passes the mammary barrier. Very few selenium determinations appear to have been carried out on milk other than that of the cow. The milk from cows grazing seleniferous areas has been shown to contain 0.3–1.2 p.p.m. Se (59), whereas milk from cows consuming normal rations contains no detectable quantities of this element.

IV. Absorption and Excretion of Selenium

1. *Absorption*

Little is yet known of the factors influencing selenium absorption in animals and apparently nothing of the main site or sites of absorption of this element within the gastrointestinal tract. From the results of numerous balance experiments and studies of the relative toxicity of different sources of selenium to laboratory animals, however, certain facts have emerged. These studies indicate that selenium is rapidly and efficiently absorbed from naturally seleniferous diets and from soluble salts of selenium added to normal diets. Thus Moxon (31) found that rats consuming a seleniferous wheat ration containing 18 p.p.m. Se, retained in their bodies 63.5% of the selenium intake within the first week. Anderson and Moxon (1) observed no difference in retention of such naturally occurring selenium and of sodium selenite administered at the same level. Smith *et al.* (54), on the other hand, using higher dietary selenium levels obtained greater retention from naturally occurring organic selenium than from sodium selenite.

The toxicity studies of Miller and Schoening (29) and of Franke and Painter (11) also imply higher absorption of selenium from cereals than from soluble selenite. These latter workers demonstrated the following order of toxicity of selenium from several sources: wheat > corn > barley > selenate > selenite > selenide > metallic selenium. The differences among the three cereals were very small but they were all definitely more toxic than the inorganic salts. Such differences in toxicity can be taken to indicate primarily, but not wholly, differences in absorption, since selenium must enter the tissues to exert its toxic effects. The relatively low toxicity of metallic selenium is a reflection of its insolubility and hence poor absorption.

The high toxicity of the organic forms of selenium present in cereal grains is of great interest but is little understood. Obviously such selenium is readily absorbed but the extent to which these compounds are broken down in the gastrointestinal tract, and the form in which the selenium actually enters the blood stream are problems awaiting solution. In this connection it should be mentioned that the toxicity to rats of a series of synthetic organic selenium compounds, including selenodiacetic acid and various selenopropionic acids, was found to be distinctly lower than that of selenite (33). Whether this difference is entirely due to lower absorption is not known.

2 *Excretion*

Excretion of selenium takes place through three channels—the breath the urine and the feces—but only at very high intakes are appreciable amounts exhaled At ordinary intakes such as induce symptoms of chronic selenium poisoning the urine constitutes the main pathway of excretion The highly odoriferous (garlic like) compound which appears in the breath of animals suffering from acute selenium poisoning has been claimed on rather unconvincing evidence to be methyl selenide (17) Rats were found to have exhaled 3–10% of a single subtoxic dose of radioactive selenium as sodium selenate within 24 hours of injection (28)

When moderate amounts of selenium are fed to laboratory animals for periods of weeks or months an equilibrium in which excretion tends to balance absorption is soon reached This suggests that a tissue saturation process is involved The time taken for this to occur varies of course with the level of intake Cats when fed sodium selenate at a uniform daily rate were found to reach such a balance within a few weeks so that 50–80% of the amount ingested was excreted in the urine and only very small amounts in the feces (54) A similar relation of output to intake was observed when laboratory animals were fed seleniferous wheat rations (31 54) though in these cases relatively less was excreted in the urine and more was retained in the tissues For instance Moxon (31) found that rats fed a seleniferous wheat ration supplying 18 p p m Se excreted in their urine 20 2–35 5% of the amount ingested over a six weeks period During this period the proportion of the ingested selenium excreted in the feces rose from 16 to 22% It appears that the amount in the feces consists largely of unabsorbed selenium since the results of injection experiments indicate that selenium once it has entered the tissues leaves the body principally via the kidneys

When the administration of selenium is discontinued the excretion level in the urine falls off rapidly in the case of inorganic compounds and rather more slowly when food selenium has been given Most of the selenium retained by rats (15) cats and rabbits (54) during the administration of inorganic selenium salts was found to be eliminated within two weeks of discontinuing treatment whether this was by injection or oral administration Anderson and Moxon (1) observed a similar rapid excretion of a high proportion of the selenium retained by rats from a seleniferous wheat ration when these animals were transfered to a nonseleniferous stock ration Most of it was eliminated within two weeks but a small proportion was retained presumably in a bound form in the tissues from which it was lost slowly over a long period

This is of considerable importance from the standpoint of public health because it is apparent that placing animals on a nonseleniferous ration for a short period before slaughter will greatly reduce the concentration of selenium in their tissues and so make them safer for human consumption The administration of compounds such as bromobenzene, which increases selenium excretion from the animal body (39), offers a further possible procedure for achieving this objective

V Selenium in Human Nutrition

The discovery of chronic selenium poisoning in animals over considerable areas of the North American continent aroused early interest in the possibilities of such poisoning in the human population consuming the food products of these areas Surveys of families living in seleniferous areas, conducted in 1935 and 1936 (51) gave definite proof that selenium was being ingested in appreciable quantities but symptoms clearly pathognomonic of selenium poisoning in man could not be obtained Concentrations of selenium ranging from about 10 to 200 μg per 100 ml of urine were demonstrated in a high proportion of the population living in the seleniferous areas, although normal human urine from individuals living outside these areas contains no selenium Attempts to correlate clinical evidence of selenium intoxication with the degree of absorption as evidenced by urinary excretion did not yield conclusive results Vague symptoms of ill health, particularly some suggestive of gastric and hepatic disorders which were believed to have been caused by the selenium ingested were nevertheless observed in some sections Cirrhosis of the liver and damage to the gastric mucosa are common symptoms of chronic selenium intoxication in laboratory animals, but positive diagnosis of this condition in man is extremely difficult in the absence of any clear evidence of the symptoms that arise as a result of continued ingestion by humans of relatively small quantities of this element over long periods Human poisoning from the ingestion of naturally occurring seleniferous foodstuffs in areas where selenium poisoning in animals is common cannot therefore be said to have been established definitely

The very different dietary habits of man and of farm stock undoubtedly provide the main reason why the former are relatively little affected by selenium in areas characterized by widespread poisoning of farm stock Cereals constitute an important item in the diet of man pigs, and poultry but the latter consume mostly whole grains and mill products such as bran and pollard which, as has been shown contain a large proportion of the selenium in wheat Man on the other hand

eats very little of these mill products or of whole grain Most of the cereal in human dietaries consists of white flour and refined cereal products that are significantly lower in selenium content than whole grain Values ranging from as low as 0 25 to 1 p p m were actually recorded for bread from seleniferous districts (51)

Milk meat and vegetables constitute the other main items in human dietaries from which selenium could be ingested when these are pro duced in seleniferous areas Examination of samples of these foodstuffs from such areas has shown that milk is unlikely to be an important source of supply Smith and Westfall (51) obtained values for milk ranging from 0 16 to 1 27 p p m Se The levels in meat from seleniferous areas were much higher namely 1 17–8 0 p p m but the range is so great that it is impossible to draw any conclusions as to the significance of this item in human dietaries The effect of cooking on the selenium content of meat is unknown All garden vegetables except onions are characteristically low in selenium as was pointed out earlier Values above 2 p p m are exceedingly rare Moreover in the cooking of vegeta bles much of the selenium is removed by treatment with water (4)

Since there are no other major items in most human dietaries and the drinking water in seleniferous areas usually contains little or no selenium it is apparent that the intakes of selenium by farm stock in these areas will normally greatly exceed those of the human population If the average limit of tolerance for man be taken as 3–4 p p m of the diet as has been claimed for animals it follows from the foregoing considera tions that this limit will be attained only under exceptional circumstances and for relatively short periods It is not surprising therefore that endemic selenium poisoning in man has not definitely been established

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CHAPTER 12

ALUMINUM, ARSENIC, BARIUM, BORON, BROMINE, SILICON, STRONTIUM, AND VANADIUM

I Aluminum

Despite its abundance in the earth's crust and in most soils and in atmospheric dust aluminum is a small and unimportant constituent of living matter It is not known to serve any useful purpose or essential function in plants, microorganisms or animals Nor, under any known naturally occurring conditions does aluminum constitute a toxic hazard to man or his domestic animals

1 *Aluminum in Plants*

Aluminum is apparently always present in variable but normally low concentrations in the tissues of the higher plants Most plants including the edible grasses and clovers contain about 15–20 p p m of aluminum on the dry basis, with greater concentrations, usually in the leaves than in the stems (72) Trees and herbaceous species have been reported to contain much higher levels of this element, of the order of 200 p p m (65), but later spectrographic studies indicate that this figure may be rather high (66) A few species particularly certain trees and ferns habitually accumulate vastly greater quantities of aluminum than those just mentioned Values of 3000 or 4000 p p m are quite common in these accumulator species (37, 86) They are not, however, normally consumed by grazing stock, so that they do not induce aluminum poisoning as selenium accumulator plants induce selenosis in animals Little is known of the mechanism involved in the absorption and retention of aluminum by such species although it has been suggested that this element may be of some value to them (38) Convincing evidence that aluminum is essential to the growth structure, or function of plants generally has not yet been produced

2 *Aluminum in Animal Tissues and Fluids*

The occurrence of aluminum in animal tissues blood and urine has been the subject of considerable controversy, arising largely from difficulties and errors in the analytical, especially the spectrographic, techniques employed It is now agreed that this element is regularly present in minute concentrations although there is less agreement on the magni

tude of these concentrations Estimations of the aluminum in the tissues of normal rats dogs and man indicate that species differences are small and that the lungs are the only body tissue carrying substantial amounts of this element The aluminum of the lungs presumably comes from atmospheric dust and not from the food or drinking water The following mean figures taken from the work of Kehoe *et al* (40a) on normal human tissues agree well with those of Myers and Mull (50) for the tissues of the dog the rat and man and with those of Wurher (90) for the dog namely kidney, 0 12 heart 0 56 brain 0 04 liver 1 6 spleen 1 3 muscle 0 15 rib bone 2 4 long bone 5 0 stomach 0 73 intestines, 0 87 and lung 59 4 p p m Al on the fresh tissue The mean value for normal whole human blood obtained by Kehoe *et al* (13 μg per 100 ml) is considerably lower than most other recorded values except those of Lewis (45) who claimed that human blood is either free from aluminum or contains only one part in five or ten million

There is no direct evidence that aluminum performs any essential functions Hove and co workers (35) attempted to produce an Al deficient diet for rats but were unsuccessful They concluded that " if aluminum is required by the rat the requirement can be met by as little as 1 μg daily " Balance experiments with children and adults give no indication that aluminum is essential in the human diet In fact Scoular (69) obtained negative balances in more than half the studies which she made with preschool children and several investigators have actually found slightly more aluminum in the feces than was ingested in the food Thus Kehoe and associates (40a) found the mean intake of aluminum from the food and beverages of a normal adult American diet to be 36 4 mg daily over 28 days and the mean daily excretion in the feces over the same period to be 41 9 mg The urine of the individual tested contained a mean of 0 05 mg per liter indicating that dietary aluminum is very poorly absorbed Similar low absorption and predominant excretion in the feces have been demonstrated for the rat rabbit and dog even when substantial dietary additions of soluble aluminum salts are made The possibility exists that at least part of the fecal aluminum has been absorbed and reaches the feces via the bile but much more evidence is needed on this point The net result of the assimilatory process nevertheless is to produce an organism very much poorer in aluminum than is the food

The interesting possibility that aluminum may be involved in the succinic dehydrogenase cytochrome c system which is the chief mechanism for oxidation of succinate in the animal body arises from the work of Horecker *et al* (36) and from that of Potter and Schneider (58)

In vitro studies by the former workers revealed that aluminum, chromium and several of the rare earths promote the reaction between cytochrome c and its reductant presumably succinic dehydrogenase Analytical evidence, both from the *enzyme preparations themselves and* from the animal body indicates that aluminum may be the element involved *in vivo* Proof of such a relationship between aluminum and this or any other enzyme system in the living animal will have to await *the development of diets and techniques capable* of inducing an aluminum deficiency state

3 *Aluminum as a Human Health Hazard*

The amounts of aluminum present in human dietaries may be increased by contamination from domestic aluminum cooking utensils and vessels used in food processing plants and by the use of aluminum sulfate baking powders Alum was at one time also used as a bleaching and improving agent in flour and bread and as a "hardener in the manufacture of pickles but this practice is now largely obsolete Extensive investigations of this aspect of aluminum have demonstrated *beyond doubt that the quantities of aluminum ingested by humans from* these sources are usually quite small and are so poorly absorbed from the alimentary tract that they do not constitute the slightest hazard to health (12) Several investigators have shown that ten times the quantity of aluminum likely to be ingested in this way can be tolerated by man without harmful effects Still larger intakes of aluminum are known to produce gastrointestinal irritation and colic and to produce rickets by interference with the absorption of phosphate (19), but the amounts required for these effects are far beyond those ever likely to occur under ordinary conditions of living Moreover therapeutic doses of aluminum hydroxide gel used in the treatment of peptic ulcer are *unlikely to reduce phosphorus absorption to dangerously* low levels because of the lower solubility of this form of aluminum compared with the sulfate (78)

II Arsenic

1 *Arsenic in Human Nutrition*

Arsenic has long been known to be constantly present in all animal tissues and fluids in consequence of its widespread occurrence in minute quantities in soils and dust and in plant and animal materials consumed as food Estimates of the total amount present in the normal human body not exposed to unusual intakes of this element vary greatly but *about 20 mg or 0 3 p p m appears to be a* reasonable figure for adults

(77). This quantity of arsenic is distributed fairly evenly throughout the body without significant concentration in any particular organ or tissue other than in the hair and nails. Normal human hair has been reported to contain 0.3–0.7 p.p.m. and nails 1.5–4 p.p.m. arsenic (80). In acute arsenical poisoning the arsenic content of the nails may increase to much higher levels. Values of 20–120 p.p.m. have been reported (39).

In ordinary human dietaries sea fish, especially shellfish and crustaceans, are the richest source of arsenic. This element rarely occurs in most foods in concentrations greater than 1 p.p.m., whereas many species of bony sea fish contain 3–4 p.p.m., oysters 5–10 p.p.m., and mussels as high as 120 p.p.m. (39). In crustaceans such as prawns the arsenic content is also usually very high. Levels up to 170 p.p.m. have been recorded (16). Fish of the same species from different localities show wide variations in arsenic content, presumably due to differences in the arsenic content of the water and the materials upon which they feed. Fish and crustaceans from fresh water, which is normally much lower in arsenic content than sea water, do not contain abnormal amounts of arsenic.

The widespread use of arsenical insecticides, particularly those containing lead arsenate, may supply appreciable quantities of arsenic to human foods unless proper precautions are taken to remove the spray residues by washing. Public health authorities in most countries now impose regulations specifying safe limits for arsenic in fruits and vegetables. Where lead arsenate spraying is practiced for long periods in orchards the surface soil may eventually become charged with arsenic to an extent sufficient to be toxic to crops, but the amounts of arsenic taken up by plants, even from such soils, are negligible (29).

For many years arsenic compounds played a major role in the treatment of syphilis, and small amounts of arsenic with iron have been used as tonics in the treatment of human anemias for centuries. Convincing evidence of its value when used in this latter way is entirely lacking. There are indications, nevertheless, that under certain conditions arsenic may be beneficial to animals, as is pointed out below. The well-known toxic effects of arsenic need not be considered here. The only reported occurrence of naturally occurring chronic arsenic poisoning, so far as is known, comes from Argentina, where several endemic foci of poisoning from arsenical waters exist in the Cordoba province (32).

2. *Arsenic and Growth*

Attempts to demonstrate arsenic deficiency in rats have so far proved unsuccessful. Hove *et al.* (33) observed no improvement in the growth

hemoglobin levels, or the number and fragility of the red cells when 1 µg or 5 µg arsenic per day was added to a mineralized milk diet supplying 2 µg arsenic daily They conclude that " if the rat requires arsenic this requirement must be less than 2 µg daily " It was found, however, that arsenic supplementation delayed the fall in hemoglobin level that is associated with removal of the rats from a diet of whole milk mineralized with iron copper, and manganese to one of whole milk alone Skinner and McHargue (74) also obtained some evidence of an effect of arsenic on hemoglobin production but none on growth Rats fed a diet composed mainly of mineralized skim milk powder and sucrose were found to respond, to a small but significant extent to arsenic supplements by increased levels of hemoglobin It should be pointed out that no such effect from arsenic was obtained when the rats were fed a mineralized *liquid* whole milk and glucose diet

A further interesting finding by Hove *et al* (33) was that about 80% of the arsenic in the blood was concentrated in the red cells In anemia a fall was observed in the arsenic content of both the cells and plasma Guthmann and Grass (30) had earlier made the intriguing claim that the arsenic content of the blood of women rises significantly during menstruation and pregnancy Normal levels of 64 µg As per 100 ml of blood were obtained, compared with almost 100 µg during menstruation and as high as 220 µg in the fifth and sixth months of pregnancy Levels above normal were also reported for the blood of cancer patients These observations led the authors to conclude that arsenic is directly related to tissue growth and cell proliferation A significant elevation of blood arsenic during human pregnancy could not be confirmed in a more recent study (70a) but a stimulation of the growth of tissue cultures (76) and of the rates of growth and metamorphosis of tadpoles (26) by minute amounts of arsenic has been reported

Several groups of workers have demonstrated a small but significant growth stimulating effect of dietary arsenic in the form of 3 nitro-4 hydroxyphenylarsonic acid (arsanilic acid), with chicks (49), turkey poults (48) and weanling pigs (13) Increased weight gains and efficiency in feed use were also reported in lambs fed a commercial mineral and vitamin supplement containing this arsenic compound (13) In a recent study (11), by contrast potassium arsenite arsanilic acid, and arsonic acid, fed to weanling lambs at levels equivalent to 0 002 0 004, 0 006, 0 012 and 0 024% of arsenic in the whole ration, gave no increase in weight gains compared with controls, either on poor or on high quality rations At these levels of arsenic no symptoms of toxicity

and no changes in the numbers of fecal coccidia trichostrongyle worms, or clostridia were observed

The pronounced effects of dietary arsenic in alleviating the symptoms of selenium poisoning and so promoting growth and well being in animals ingesting toxic levels of selenium were discussed in Chapter 11

3 *Arsenic and Thyroid Function*

The antagonism between arsenic and selenium is paralleled, to some degree by an antagonism between arsenic and iodine Numerous reports of an arsenic thyroid antagonism have appeared (32), to which brief reference was made in Chapter 9 (iodine) Sharpless and Metzger (71) carried out critical experiments with rats which unequivocally established a goitrogenic role for arsenic When fed in nontoxic amounts (0 005% of diet) arsenic was shown to have a slight but not significant goitrogenic effect At a level of 0 02% arsenic decreased the growth of rats approximately 50% reduced the iodine concentration in the thyroid, and significantly increased the weight of this gland Providing five times the minimum requirement of iodine with this level of arsenic reduced but did not eliminate the goitrogenic and the toxic effects It was calculated that 0 02% arsenic in the diet more than doubled the iodine requirement

Arsenic is known to have a retarding effect on several redox systems probably by combining with sulfhydryl groups (1) It could be argued that the goitrogenic action of arsenic is due to a compensatory response by the thyroid to the reduced oxidative capacity of the tissues produced by the arsenic A satisfactory explanation of this action of arsenic must however await further experimentation and a better understanding of the action of the thyroid hormone itself at the intracellular level Meanwhile it should be appreciated that the goitrogenic action of arsenic is negligible where this element is ingested in nontoxic amounts and where iodine intakes are normal In man a goitrogenic effect from arsenic can only be anticipated where the iodine intake is low and the arsenic intake sufficient to be slightly toxic It is significant that a high incidence of goiter occurs in the Styrian Alps the home of the arsenic eaters and in the Cordoba Province of Argentina mentioned above where chronic arsenical poisoning is endemic (32)

III Barium and Strontium

Barium and strontium can conveniently be considered together because of the frequency with which they have been investigated together although they differ greatly in their distribution in living matter Both

elements are commonly present in low concentrations in plant and animal tissues The range of strontium in animal tissues, according to Gerlach and Muller (24), is 0 01–0 10 p p m and there is no evidence of accumulations of this element in any particular species, organ, or tissue Barium has been shown to occur constantly in the choroids of cattle and other species (61) and remarkably high *levels have been* demonstrated in Brazil nuts (64 70) The amounts in Brazil nuts vary with the locality in which they are grown, but levels of 3000–4000 p p m of barium are common (64 70) These accumulations of barium are not accompanied by unusually high concentrations of strontium In general, the strontium content of living tissues is significantly higher than the barium content Sea water contains 10 p p m strontium compared with only 0 5 p p m of barium (27)

Barium and strontium have been reported to act as plant growth stimulants (68) but neither element has been shown to be essential to the growth or development of plants Both are absorbed from the food and are retained in the body to an exceedingly small extent, particularly in the bones (82)

Within the last few years highly suggestive evidence that barium and strontium are essential in the diet of rats and guinea pigs has been produced Rygh (67) fed these animals on diets highly purified by special techniques When such diets were supplemented with a "complete mineral mixture, growth and tooth and bone development were *satisfactory The omission of either barium or strontium from* the mineral supplement caused a depression in growth The absence of strontium resulted, further in a serious impairment of the calcification of the bones and teeth and a higher incidence of carious teeth than occurred in the positive control animals (Similar results on the calcification of the bones and teeth were obtained with vanadium These are discussed in Section VII of this chapter) The position with barium was quite different In contrast to strontium, evidence was obtained that this *element instead of promoting mineralization effected* decalcification of the osseous tissues It seems, therefore that barium and strontium should tentatively be added to the list of essential trace elements in animal nutrition although confirmation of the important researches of Rygh is needed before such an assertion can be made with complete conviction

IV Boron

Although boron was one of the first of the trace minerals found to be essential for plant life and a very considerable literature exists on its distribution in living organisms and its functions in the higher plants there is no positive evidence that it performs any useful or essential function in animals

1 *Boron in Plants*

It is almost a century since boron was first shown to be present in plants (89) and by 1895 its universal distribution throughout the plant kingdom was established (40) Incontrovertible evidence that this element is essential to the higher plants was obtained in 1923 (85) and confirmed and extended during the following four years (10) Crop responses to borate applications were demonstrated in many parts of the world during the ensuing decade and indications were obtained that boron functions in the transformation and utilization of carbohydrates and in respiratory processes

The boron content of plants varies with the species the part of the plant the maturity of the plant and the available boron in the soil Legumes normally carry higher concentrations of boron than grasses or cereals and the leaves are generally richer in this element than the roots stems or seeds In the compilation of Beeson (3) green clovers and legumes are the plant materials richest in boron (25–50 p p m on the dry basis) cereal grains and hays are the poorest (1–5 p p m) and most fruits and vegetables are intermediate (5–20 p p m) Variation among samples of the same species is considerable owing principally to differences in the available boron in the soil

2 *Boron in Animal Physiology*

Since boron is a constant constituent of plant tissues it is an inevitable component of human and animal diets and finds its way into all parts of the animal body Bertrand and Agulhon (7) as early as 1912 detected boron in small amounts in all of the many materials they examined and postulated its presence in all animal tissues Muscle tissue and most of the organs of the body generally contain less than 1 p p m boron but the amounts are raised markedly following the ingestion of or treatment with toxic amounts of boric acid (57) The highest concentration of boron under these conditions occurs in the brain followed by the liver and the body fat in that order Whether this unusual distribution in which higher levels occur in the brain than in any other body organ or tissue exists in the normal animal is unknown

Cow's milk normally contains 0.5–1.0 p.p.m. of boron with little variation according to breed and stage of lactation (34, 54). The concentration can readily be raised by supplementing the cow's diet with borax or boric acid. Thus Owen (54) found that the addition of about 20 g borax daily to the ration increased the boron content of the milk from 0.7 to over 3 p.p.m. Boron is also transmitted to the egg when the hen's diet is supplemented with additional soluble forms of boron.

No function for the boron present in the animal body has so far been revealed. Several unsuccessful attempts have been made to produce boron deficiency in rats by the use of highly purified synthetic diets containing only 0.15–0.16 p.p.m. boron in the dry matter of the whole ration (31, 53, 81). The rats on these diets, which supplied no more than 0.6 μg B per rat per day, grew normally, reproduced and reared young which appeared normal. Boron supplements produced no discernible improvement in these animals. It appears that if boron is required by the growing rat it must be at a level below 0.15 p.p.m. of the dry ration.

A possible relationship between boron and potassium has been investigated by Skinner and McHargue (73) and by Follis (22). Potassium deficient rats were found by the former workers to survive longer when boron supplements were added to the rations, and after 21 days the rats receiving the boron had more glycogen in their livers and better stores of body fat than the controls receiving no supplementary boron. On the other hand, Follis found that boron additions had no effect on the growth rate or survival time of potassium deficient rats or on the heart and kidney lesions associated with potassium deficiency. The question of a possible connection between boron and potassium in the animal organism clearly requires further study in which the food consumption of the test and control animals is properly controlled.

The average intakes of boron by human adults from ordinary diets range from about 10 to 20 mg per day (41). The higher amounts are associated with the consumption of large amounts of fruit. As indicated earlier, fruits and vegetables are very much richer in boron (5–20 p.p.m.) than cereal grains or flesh foods and milk (0.5–2 p.p.m.). They therefore constitute the principal source of this element in human dietaries (8). The boron in food and boron added as soluble sodium borate or boric acid is rapidly and almost completely absorbed and excreted mostly in the urine (41, 54). Where very high intakes of boron occur, either accidentally or from the treatment of large burns with boric acid, absorption is also rapid and excretion takes place largely in the urine. Sufficient amounts may be retained in the tissues, however, especially in

the brain to produce serious toxic effects before elimination by the kidneys can reduce the concentrations to innocuous levels (57)

V Bromine

Bromine is an exceedingly interesting trace element because of its chemical relationship to iodine and of its surprising richness in living tissues compared with iodine Whether the presence of bromine in living tissues is merely fortuitous owing to passive ingestion from soils waters and foods or whether it has some physiological function is unknown Very recent experiments suggest that it can act as a stimulant to seed radicle growth (9) but incontrovertible evidence that it plays a vital role in any living process has not yet been obtained

1 *Distribution of Bromine in Plant and Animal Tissues*

Examination of plant and animal materials shows that the food regularly supplies considerable but highly variable quantities of bromine to the human body The drinking water may also supply appreciable amounts of this element Much less is known of bromine intakes by farm animals but owing to its constant presence in plants and the relatively high levels of this element in animal tissues substantial quantities must also be ingested and absorbed by all farm stock In general 10 to 100 times as much bromine as iodine occurs in foods and water Thus Damiens and Blaignan (17) give the bromine content of bread as 0 9–6 1 p p m and Ford *et al* (23) give values ranging from 5 2 to 7 9 p p m for untreated white flour Similar levels of bromine in white flour (5 2 and 7 9 p p m) have been reported by Winnek and Smith (88) who also found 40–42 p p m in milk powder and 94 p p m in egg albumin Marine plants are usually much richer in bromine than land plants and the cereal grains (1–11 p p m) are among the lowest of all materials commonly used as food (51) Common salt can be a rich source of bromine One sample of salt was found to contain no less than 1 mg bromine per g chlorine (20)

Animal tissues are normally 50 to 100 times richer in bromine than they are in iodine with the exception of the thyroid gland for which the reverse holds Differences among species at least for the rat the pig and man are small and bromine is not especially concentrated in any tissue or organ so far examined (20 83 88) The levels in the tissues *are markedly influenced by the level of intake of bromine* either in the diet or as added bromide and by the bromine chlorine ratio of the diet (see Table 47) There is an interchange between bromide and chloride in the body fluids and tissues so that the administration of

Cows milk normally contains 0 5–1 0 p p m of boron with little variation according to breed and stage of lactation (34 54) The concentration can readily be raised by supplementing the cows diet with borax or boric acid Thus Owen (54) found that the addition of about 20 g borax daily to the ration increased the boron content of the milk from 0 7 to over 3 p p m Boron is also transmitted to the egg when the hens diet is supplemented with additional soluble forms of boron

No function for the boron present in the animal body has so far been revealed Several unsuccessful attempts have been made to produce boron deficiency in rats by the use of highly purified synthetic diets containing only 0 15–0 16 p p m boron in the dry matter of the whole ration (31 53 81) The rats on these diets which supplied no more than 0 6 µg B per rat per day grew normally reproduced and reared young which appeared normal Boron supplements produced no discernible improvement in these animals It appears that if boron is required by the growing rat it must be at a level below 0 15 p p m of the dry ration

A possible relationship between boron and potassium has been investigated by Skinner and McHargue (73) and by Follis (22) Potassium deficient rats were found by the former workers to survive longer when boron supplements were added to the rations and after 21 days the rats receiving the boron had more glycogen in their livers and better stores of body fat than the controls receiving no supplementary boron On the other hand Follis found that boron additions had no effect on the growth rate or survival time of potassium deficient rats or on the heart and kidney lesions associated with potassium deficiency The question of a possible connection between boron and potassium in the animal organism clearly requires further study in which the food consumption of the test and control animals is properly controlled

The average intakes of boron by human adults from ordinary diets range from about 10 to 20 mg per day (41) The higher amounts are associated with the consumption of large amounts of fruit As indicated earlier fruits and vegetables are very much richer in boron (5–20 p p m) than cereal grains or flesh foods and milk (0 5–2 p p m) They therefore constitute the principal source of this element in human dietaries (8) The boron in food and boron added as soluble sodium borate or boric acid is rapidly and almost completely absorbed and excreted mostly in the urine (41 54) Where very high intakes of boron occur either accidentally or from the treatment of large burns with boric acid absorption is also rapid and excretion takes place largely in the urine Sufficient amounts may be retained in the tissues however especially in

the brain to produce serious toxic effects before elimination by the kidneys can reduce the concentrations to innocuous levels (57)

V Bromine

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bromide results in the replacement by bromide of a proportion of the chloride of the body, thus increasing the bromine concentrations present (47) Conversely, the administration of large amounts of chloride accelerates the elimination of bromide, thus reducing the bromine concentrations present Dosing with chloride has been recommended as a treatment for bromide intoxication for this reason

TABLE 47

EFFECT OF BROMINE INTAKE AND BR CL RATIO ON THE BROMINE CONTENT[a] OF THE TISSUES OF RATS (88)

Tissues	Stock diet 20 p p m Br (Br Cl 0 009 1)	Synthetic diet + KBr 20 p p m Br (Br Cl 0 021 1)	Synthetic diet 0 5 p p m Br
Whole blood	117–130	336–503	2.8–14
Hair and skin	41–47	90–170	3 5–5 4
Liver	26–29	39–130	2 2–3 5
Muscle	13–17	41–62	2 9–6 2
Kidney	65–70	220–230	7–20
Spleen	54–56	160–190	8–32
Brain	25–26	82–89	3 3–21
Young at birth	85–105	320–410	7 3–14

[a] Measured in p p m on the dry basis

The only attempt to produce a bromine low ration and to test its effects on animals, so far as is known, is that of Winneck and Smith (88) These workers prepared a specifically purified diet containing less than 0 5 p p m bromine On this diet rats made reasonable growth over a period of 11 weeks showed no signs of ill health, and produced apparently normal young A similar group receiving the same basal diet plus 20 p p m bromine as KBr made no better growth and did not differ in appearance or reproduction records from the control animals The amount of bromine in the young rats at birth was however, very much lower in the unsupplemented group It is interesting to speculate upon the possibility of a bromine deficiency arising in the second generation on such a bromine low diet when the bromine stores might be still further depleted For the present it can be stated that either bromine is nonessential or it is required by the rat at less than 0 5 p p m in the diet

2 *Bromine and Mental Disease*

A great stimulus to investigation of the physiology of bromine was given by the work of Zondek and Bier (91) in which the claim was made that the blood of depressive psychotics contains *subnormal* con

centrations of bromine A further impetus to such work was provided by the claim of Bernhardt and Ucko (5) that the pituitary gland contains fifteen to twenty times the bromine content of blood and that a bromine containing hormonelike organic compound is stored in the pituitary and circulates in the blood causing sleep and a sedative effect Support for these claims was given by several subsequent investigations The development of improved methods for the determination of bromine has provided abundant evidence which completely invalidates the earlier work The Pincussen and Roman method used by these earlier workers was shown to be unreliable and subject to gross errors The bromine content of the pituitary is not significantly higher than that of blood (20 83) there is no evidence for the existence of an organic bromine compound in blood (20 59) and the bromine content of blood is not significantly below normal in manic depressive states (14 20) Thus Dixon found the mean bromine content of 10 normal human blood samples to be 0 73 mg per 100 ml (range 0 39–1 36) and that of 12 manic depressive psychotic patients to be 0 77 mg per 100 ml (range 0 72–1 72) Using a very delicate micromethod Conway and Flood (15) obtained a lower mean figure for normal human blood namely 0 37 mg bromine per 100 ml

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Although it provides a very obvious field for investigation a possible relationship between bromine and iodine metabolism has attracted very few workers In fact it is not even definitely established that the normal thyroid contains more bromine than any other tissue in the body Perlman and associates (56) studied the distribution of administered radioactive bromine and concluded from their data that the thyroid showed a greater uptake of the labeled bromine than any of the other tissues tested and that this uptake could not be explained on the basis of simple diffusion from the blood serum Baumann and co workers (2) could find no more bromine in the normal thyroid of rabbits than in the blood but glands which were hyperplastic owing to a relative or absolute deficiency of iodine were found to be significantly richer in bromine than the blood These workers argue that thyroid tissue can distinguish only imperfectly between bromine and iodine so that in the absence of iodine it seizes some bromine in its place When iodine is supplied to animals with hyperplastic thyroids they quickly lose the bromine which was accumulated in a futile effort to overcome the lack of iodine Apparently the bromine accumulated in this way cannot be utilized by the thyroid to synthesize a hormone No inhibition of the development of iodine

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Tissues	Stock diet 20 p.p.m. Br (Br:Cl 0.009:1)	Synthetic diet + KBr 20 p.p.m. Br (Br:Cl 0.021:1)	Synthetic diet 0.5 p.p.m. Br
Whole blood	117–130	336–503	2.8–14
Hair and skin	41–47	90–170	3.5–5.4
Liver	26–29	39–130	2.2–3.5
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deficient goiters in rats could be obtained by Richards *et al* (62) by the administration of large amounts of bromine as NaBr, although KI was completely effective It seems that the thyroid is incapable of the brominating process The bromine analogue of thyroxine (tetrabromo thyronine), however has been shown by several workers to have, in several animal species the same qualitative effect as thyroxine although in much lower degree In the treatment of human myxedema tetra bromothyronine is one fifteenth to one sixtieth as active as thyroxine in the prevention of the development of goiters (44) Since the correspond ing chlorine analogue of thyroxine (tetrachlorothyronine) also has some thyroxinelike activity, although of a still lower order, it is clear as was pointed out in Chapter 9 that the physiological action of thyroxine is not completely dependent upon the presence of iodine in the thyronine nucleus

VI Silicon

1 *Silicon in Plant and Animal Tissues and Fluids*

Silicon resembles aluminum in its high concentration in soils and at mospheric dust It differs markedly from aluminum however, in being present in most plants particularly gramineous species in comparatively large amounts and in animal tissues and fluids in very much higher concentrations than aluminum or indeed, than a majority of the trace elements

Silicon has long been known to constitute a considerable percentage of the ash of plants In grasses and cereals this may amount to 30–40% or more of the ash or 3–4% of the whole dry plant In clovers and herbs the proportion is usually appreciably lower A great deal of this silicon is present in the cell walls which no doubt accounts for much of the early work now largely discounted which attempted to demonstrate a relation between the silica content of plants and the strength of the tissues and the liability of cereals to lodging The silicon of plants is apparently partly present as insoluble silica partly as soluble silicates and partly in organic combination This has been demonstrated in recent studies of oat straw (87) About 82% of the silica in this material was found to be extracted by hot water or methyl alcohol The re mainder was firmly bound to the cellulosic structure of the cell and could not be extracted without destroying the cellulose Evidence was obtained also of the presence of organic silicon complexes containing galactose and a pentose (87)

Many years ago it was concluded that silicon is essential for the re

production of marine plankton (63) and it is now generally accepted that this element is essential in the nutrition of the higher plants In culture solutions designed to exclude silica rigidly from the nutrients the glass containers and the atmosphere improved growth from additions of silicates has been demonstrated with rice and millet (75), with sun flower and barley (46) and with beets (*Beta vulgaris*) (60) Little or nothing is yet known of the mode of action of silicon in plant growth or metabolism

The normally high silica content of soils plants and the atmosphere ensures a continuous high intake of this element by all animals although no indication of an essential function for silicon in the animal body has been brought forward or even foreshadowed Nearly forty years ago Gonnerman (28) carried out numerous analyses of animal tissues and foodstuffs as a result of which he concluded that silicon is of some im portance in the nutrition of man and the higher animals For instance he found silica to constitute up to 77% of the ash of feathers and sug gested that it played some part in maintaining their rigidity The de velopment of modern micromethods of analysis has shown that much of the early work on the distribution of silicon in the animal body gave values much too high Even fetal tissue however has been shown to contain appreciable quantities the normal range being about 40–400 p p m expressed as SiO on the dry tissue compared with 50–1000 p p m for normal adult human tissues (42) The highest levels in the adult are found in the lungs presumably owing to the inhalation of dust as in the case of aluminum and the lowest are found in the muscles In the fetus on the other hand the lungs are the lowest in silica and the muscles the highest of the tissues examined (42) The blood of man and other species contains an average of 0 5 mg Si per 100 ml This level is not increased by the inhalation of silica dust by ingestion in the food or even by injection (42 43) The silica content of the wool of sheep is also apparently unaffected by the silica content of the diet (55)

It appears that the body has a very low renal threshold for silicates because considerable amounts of silicon are absorbed from ordinary diets and excreted in the urine The amounts of silica so excreted can be greatly increased by raising the levels of silicates in the diet This contrasts greatly with the position for aluminum which is exceedingly poorly absorbed The possibility that silicon may play some part in the acid base equilibrium of the animal body has been suggested (43) but there is no proof of this

2 *Siliceous Urinary Calculi*

Sheep and cattle ingest exceptionally large quantities of silica, an appreciable proportion of which is absorbed and excreted in the urine Herbivorous animals have been shown to excrete 10–30 times as much silica in their urine as carnivorous species (43) No less than 150–200 mg SiO_2 is commonly excreted daily in the urine of sheep (4, 52) There is considerable individual variation in the amounts excreted daily but the concentrations vary even more than the total amounts because of fluctuations in urine volume Very little is yet known of the dietary or other factors which determine the magnitude of the silica excretion It is apparently a very complex process in which the level of silica (or silicate) ingestion is but one of the factors involved (4, 79)

Normally the urinary silica is eliminated harmlessly, and more or less completely either in true solution as silicate or in colloidal dispersion In some animals, however especially castrated males (steers and wethers), a portion of the silica is deposited as granules in the kidneys bladder or urethra to form calculi or 'stones These may consist very largely of silica, usually with some proteinaceous material together with variable amounts of other minerals, particularly calcium and magnesium phosphates (79) Such urinary calculi are capable of blocking the passage of urine, causing water belly, and resulting in the death of the animal This condition is seasonal in occurrence and rarely affects more than a small proportion of flock or herd It is nevertheless, a serious stock problem in some parts of the world in some seasons

Attempts to produce urinary calculi experimentally have proved unsuccessful practically without exception Neither adding silicates to the diet nor restricting the water consumption of steers or wethers has resulted in the production of siliceous urinary calculi and various mineral imbalances have yielded inconclusive results Beeson *et al* (4) obtained some results pointing to the possible importance of urinary magnesium but their results do not appear to have been carried further These authors state, " the data at hand permit the postulate that at times the solubility limits of the silica in the urine become exceeded due to hyperexcretion and small urine volumes so that silica is deposited as calculi in the kidney structure The amount of magnesium probably exerts a strong influence in the determination of the silica solubility limits Swingle (79), on the other hand believes that the development of these calculi can possibly be explained by the formation of insoluble silicic acid protein complexes in the urine although the nature of the conditions favoring the formation of these complexes in some animals and not in others remains unexplained

Of great theoretical interest is the recent finding that siliceous urinary calculi in steers respond to injections of the enzyme hyaluronidase (21) Such injections are known to be effective in relieving a colloid shortage in human urine and are used with some success in the treatment of human kidney stones This suggests that the silica in the urine of normal animals is held in solution or suspension by protective colloids, which are deficient in the animals that develop calculi Much further investigation is necessary however, before even this point can be established It is clear that the disturbance in silica metabolism that results in the formation of calculi is one of great complexity which will not readily yield to investigation

VII Vanadium

Vanadium is widely distributed in minute concentrations in soils and plants According to Vinogradov (84) most terrestrial and marine plants contain about 0 1 p p m Bertrand (6) found the higher average of 1 p p m, with a range of 0 27–4 2 p p m for the dry matter of a wide range of plants Lower concentrations were found by this worker in the organs and tissues of vertebrate animals By means of a spectrographic method with special vanadium free graphite electrodes capable of detecting 1–5 p p m vanadium in the ash of biological materials Daniel and Hewston (18) were unable to detect vanadium in a number of foodstuffs or in eggs or in the blood or tissues of normal rats Lowater and Murray (46a) on the other hand found traces of vanadium in human teeth and drew attention to the fact that this metal is isomorphous with phosphorus and can replace it in the apatite molecule This latter point is of considerable interest in the light of findings detailed below, indicating a caries inhibiting function for this element

There is some evidence that vanadium in small quantities stimulates *plant growth and that it resembles molybdenum in promoting growth* and activation of Azotobacter but until recently the only satisfactory evidence of an essential biological role for this element was the demonstration of its presence in high concentration in the respiratory pigment of several Ascidiae (31) This unique vanadium protein compound differs from hemocyanin and hemoglobin in being incapable of combining with molecular oxygen but there seems little doubt that it is related to cellular oxidation processes in these organisms

During the last few years highly interesting and suggestive evidence implicating vanadium in the mineralization of osseous tissues has been obtained In the experiments of Rygh (67) with rats and guinea pigs fed specially purified diets which were mentioned above in connection

with barium and strontium, it was found that vanadium (as well as strontium) markedly promoted mineralization of the bones and teeth during the development of the animals. Moreover, the largest number of carious teeth was observed in the vanadium- (and strontium-) deficient animals. The results of Rygh's experiments stimulated Geyer (25) to test the effect of vanadium upon animals fed a standard caries-producing diet. The numbers of animals (Syrian hamsters) used by Geyer were unfortunately small, although he states that the series of tests were repeated several times, but the results were remarkable. The addition of vanadium pentoxide (V_2O_5) to the caries-producing diet that had been fed for 21–24 days at the rate of 0.08 mg. or 0.04 mg. daily or injected subcutaneously at the rate of 0.07 mg. once a week induced a marked reduction of new enamel caries and a cessation of progress of dentin caries. From theoretical considerations the author suggests that vanadium ions embedded in enamel and dentin could increase the hardness of the hydroxyl apatite as well as the cohesion between the organic and inorganic matter. Further investigation of the relation of vanadium to calcification of bones and teeth and its possibilities in the human caries problem are clearly invited.

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CHAPTER 13

SOIL PLANT ANIMAL INTERRELATIONSHIPS

Both plants and ultimately man and his domestic animals depend upon the soil for their supply of all mineral nutrients The relationship between the plant and the soil is a direct one and is simplified by the fact that the plant is stationary Animals on the other hand because of their mobility may derive their nutritive requirements from a variety of different types of soil In addition appreciable amounts of some minerals may be obtained from the drinking water which is not necessarily derived from local soils but from deep seated rock formations or far distant areas

In ancient times in the older settled countries or in the early days of colonization of the western world animals were relatively free to roam over wide areas which generally enabled the disabilities associated with particular soils to be avoided or sufficiently offset to prevent any serious consequences to the stock Growing population and intensification of settlement steadily imposed a restriction on movements until inevitably some animals became dependent upon a narrower range of soil types certain of which were incapable of sustaining health or thrifty condition in the animal The fact that animals would not thrive or suffered various disorders in some areas whereas on other often adjacent areas no such disabilities were apparent was recognized and reported upon in many parts of the world as early as the eighteenth century Characteristic of such conditions were the interspersion of normal and abnormal areas and the readiness with which transfer of animals from an abnormal or affected area to a normal or healthy area tended to prevent or overcome the malady These observations naturally turned attention to the relation of the disorders to the soils on which they occurred

Investigations carried out during the last thirty years which have been described in earlier chapters and extending over an extraordinarily wide geographical range have revealed that many such maladies are nutritional disorders which result primarily from the inability of the soils of affected regions to supply through the plants which grow upon them the essential mineral needs of animals in adequate amounts or proper proportions The naturally occurring nutritional diseases of animals which have been related to soil characteristics in this way may be divided for convenience into two general classes—those due to a

deficiency of one or more mineral elements, such as iodine copper, and cobalt deficiencies and those due to an excess of one or more minerals such as selenium or molybdenum toxicities A feature of some of these problems is that they are not caused by simple deficiencies or excesses of a single element They may be conditioned or accentuated by other factors, particularly by the extent to which other elements or nutrients are present or absent, from the diet These other factors have also been shown to be primarily a reflection of the soils on which the herbage is grown

I Soil Relations in Human Nutrition

Nutritional deficiencies or excesses in man are more difficult to relate to soil causes than they are in farm stock because of the variety of foods in modern dietaries and the wide range of localities from which an increasing proportion of the diet is derived Endemic goiter is the outstanding and in fact the only important example of such a relation ship in man Subnormal levels of iodine in the soils, and hence in the foods and waters of certain parts of the world can be correlated with the incidence of goiter in both man and stock in those particular areas In New Zealand, Hercus and co workers (4) have stated that variation in the average amount of iodine in soils containing more than 10 p p m has little effect on the small incidence of goiter, but as the amount of soil iodine decreases so the incidence of goiter rises (see Fig 29) Even with endemic goiter there is some evidence of a decline in incidence associated with transportation of foods higher living standards and

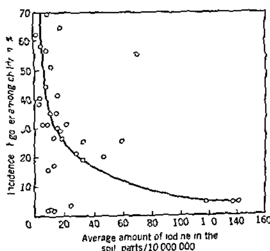


FIG 29 Relation between the average amount of iodine in the soil and the regional incidence of goiter among schoolchildren in New Zealand (As presented by Beeson (1) from the data of Hercus *et al* 3a)

greater diversity of diets as well as with the introduction of iodine prophylaxis In parts of Florida also a nutritional anemia in children has been correlated with low iron intakes due to the consumption of exceedingly poor quality diets containing a high proportion of local foods grown on poor leached mineral deficient soils

Further in the seleniferous areas of South Dakota and neighboring states " a high incidence of symptoms pointing to gastric or intestinal dysfunction and a few instances of apparent hepatic dysfunction both probably the result of continual selenium ingestion have been reported (7) It is not certain as pointed out in Chapter 11 that these conditions in man are definitely due to selenium poisoning although the evidence is highly suggestive Symptoms of chronic fluorine toxicity in man have also been observed in certain parts of the world, due to the consumption of naturally fluorided waters

It must be emphasized however that trace element deficiencies in man are far less serious and much more restricted than they are in grazing or stall fed animals For instance no evidence of copper deficiency in man has ever been recorded although extensive areas of copper deficient soils exist in which both the crops and stock are affected Differences between man and his domestic animals of this kind can be explained on the basis of an interaction between a number of contributing factors the relative significance of which varies with the particular conditions and the particular element in question These factors are (a) lower minimum requirements—this being merely an example of well known species differences in trace element needs (b) wider sources of supply involving the consumption of foods from a range of soil types (c) greater variety of foods in the dietary so that trace element abnormalities in particular parts of plants or animal tissues and fluids tend to be offset by the consumption of other food materials not so affected and (d) greater opportunity for contamination with adventitious sources of trace elements during storage preservation and especially processing so that many human foods may be appreciably higher in some elements notably iron copper zinc tin and nickel when actually consumed than when originally produced

When these factors are taken into account it seems hardly surprising that with the conspicuous exception of iodine trace element intakes are so much less readily related to the soil and nutritional disabilities associated with these elements are so much less obvious in man than they are in farm stock It is possible that a subtle relationship exists between the well being of groups of people and the soils from which their food is derived but apart from the incidence of endemic goiter

and the possible selenosis mentioned earlier such a relationship has yet to be clearly established Nutritional disabilities of man are far more likely to be associated with a poor *choice* of the foods consumed than with their source Where the choice of foods is poor, so that the diet is of low nutritional value anyway, as in the Florida survey mentioned earlier, soil deficiencies are likely to accentuate the position and even to precipitate frank deficiency symptoms But for the overwhelming bulk of mankind a diet well balanced and nutritionally adequate in other respects is likely, on present evidence to provide the normal individual with an abundance of all the trace elements with little chance of deleterious excess

II Factors Influencing the Trace Element Contents of Plants

The concentration of trace elements in plants the most important quantitative source of food to man and animals, is primarily dependent upon (a) the species and (b) the nature of the soil on which they are grown Other factors such as climate season and stage of growth can be significant in some instances and the treatment given to the soil or the plant by man such as the application of fertilizers or other soil amendments, or plant pest and disease control materials may have profound effects But basically the mineral composition of the plant is a reflection of its species and the soil conditions under which it has grown

Generic or species differences in the major mineral contents of plants growing under the same soil conditions have long been known The strikingly higher calcium content of clovers and other legumes than that of grasses is the classic example of this Data for the trace elements are less extensive but they are sufficient to establish that significant generic species and even strain or varietal differences exist in the capacity of plants to absorb various trace elements from particular soils and in the concentrations that they normally carry in their tissues This question was very thoroughly reviewed by Beeson (1) in 1941 Legumes generally contain higher concentrations of most trace minerals, including cobalt copper manganese and zinc than do grasses and most pasture herbs are also normally higher in trace elements than grasses Within the grasses significant differences in the concentrations of cobalt copper and manganese have been demonstrated when these grasses are grown on the same soil type and sampled at the same growth stage (2) Even in the seeds of cereals significant and important differences in the average concentration of at least one trace metal namely manganese have been clearly established as was made clear in Chapter 8 Certain species also have the inherent capacity to accumulate unusually high

concentrations of certain elements in their tissues Striking examples of this are the selenium loving *Astragalus* species whose presence in certain areas is a potent factor in the incidence of selenosis in grazing stock in those areas and the range of species which normally accumulate extraordinarily high concentrations of aluminum (5)

Particular aspects of this problem were considered earlier for individual elements in the appropriate chapters but it is obvious that the botanical composition of herbage can be an important consideration in determining the incidence of those nutritional disabilities in animals which are related to deficiencies or excesses of the trace elements In deficiency areas some amelioration of the deficiency could be expected by the encouragement where possible of plants with an inherently high capacity to absorb the deficient element from the soil and conversely in toxicity areas by the elimination of plants with an inherently high absorptive capacity The possibilities of such changes are limited, however, especially in deficiency areas because a plant that normally carries a relatively high internal concentration of a particular element may "adjust" itself to a deficient soil simply by reduced growth

The effect of soil differences upon the trace element concentrations in plants is in practice easily the most important of the various influencing factors All over the world various nutritional diseases and unthriftiness of stock have been correlated with particular soil types as was mentioned earlier although these soil types may vary greatly from one place to another *even with the same trace element deficiency or excess* Thus cobalt deficiency conditions in ruminants have been reported on podsolized granitic sands on erupted volcanic soils and on highly calcareous sands copper deficiency occurs in grazing stock on sands, on peats or "mucks" and on marls and iodine deficiency (endemic goiter) occurs in man and animals on sandy on alluvial and on limestone soils Rarely have any such deficiencies been observed on soils of "heavier" texture rich in the clay minerals and organic matter and derived from basic rocks

A great deal of this apparent complexity has been resolved by recent investigations in geochemistry soil chemistry and plant nutrition It is now apparent that the capacity of plants to absorb an element from a given soil is markedly influenced by the total concentration of that element in the soil and by its availability that is its chemical form or combination in the soil which in turn may be greatly affected by the extent to which other elements are present and by the soil reaction or pH

In regard to the former laws of geochemical affinity have been established by Goldschmidt (6) Carroll (3) and others which permit a

prediction of the relative liability to richness or paucity of certain elements in rocks and in the soils derived directly from them The dark ferromagnesian minerals (amphiboles, pyroxenes biotites and olivine) act as hosts to traces of such elements as copper cobalt, and zinc which occur in the crystal lattice structure of these minerals Soils derived from acid rocks low in these minerals such as granites and granite gneisses, are therefore liable to be deficient in these trace elements and should be regarded with suspicion Such effects of the nature of the parent rock on the composition of the soil are basic but it is important to appreciate that the quality of a soil is the product of a complex of climatic factors operating over long periods which may greatly influence trace element concentrations In particular, highly leached (podsolized) soils are liable to have lost an appreciable proportion of these elements Where such leaching has taken place on soils derived from acidic rocks low in the trace element bearing minerals deficiencies affecting plants and animals can be predicted with confidence It is in fact, on such soils that typical copper and cobalt deficiencies occur in Florida and in south western Australia

Effective consideration of the many factors influencing the availability of trace elements in soils, and hence the growth and composition of plants, clearly lies outside the scope of this text, although they are of the utmost importance to the whole question of soil plant animal inter relationships The reaction or pH of the soil is especially significant with a number of the trace elements Manganese deficiency in plants for instance appears invariably to be associated with a lack of readily reducible manganese oxide in alkaline soils rather than with a lack of total manganese Molybdenum also is not absorbed readily by plants from acid soils Teart soils which carry pastures exceptionally high in molybdenum are nearly all derived from the clays and limestones of Lower Lias formations and are calcareous and alkaline in character By contrast molybdenum deficiency in plants and hence low levels of molybdenum in the herbage usually occur on soils whose reaction is acid or at most neutral

These considerations suggest two major means of raising the uptake of particular elements by plants in deficiency areas and so ensuring an adequate intake of such an element by animals These are (1) the application of fertilizers containing the element in question and (2) the use of soil amendments such as lime or sulfur to raise or lower respectively, the soil pH Both have their uses depending upon the particular conditions and upon the element but the former is almost universally applied to overcome deficiency problems in grazing stock

Treatment of copper or cobalt deficient soils, for instance with copper ized" or "cobaltized" fertilizers is generally a highly effective means of increasing the concentrations of copper or cobalt in the plants growing on these soils In this way the concentrations are raised to normal" or adequate levels from the point of view of the stock depending upon the plants for their nutriment Numerous experiments have established that the use of iodine containing fertilizers is similarly effective in raising the iodine content of vegetables and other plant products used as human foods in goitrous areas but such treatment is very difficult to control both because of the highly variable uptakes of iodine by different plants and from different soils and because of the varying proportion of the different classes of foods consumed by different individuals In the treatment of endemic goiter therefore it is usual to by pass the plant and the soil and to raise the iodine intakes of man and beast directly by the use of iodized salt

III Qualitative and Quantitative Differences in the Trace Element Requirements of Plants and Animals

A further aspect of soil plant animal interrelationships that is of considerable interest and practical importance is the great qualitative and quantitative differences between the trace element requirements of plants and animals and within plants and animals This was referred to briefly in Chapter 1 Very marked differences exist among the various trace elements in this respect Thus soils that are subnormal in total or available iodine produce plants which are subnormal in iodine content but this is the only measurable response of the plants to such soils There is no reduction in growth and there are no symptoms of disease The low iodine levels in the plants growing on these soils are therefore of no significance to the plants themselves but are of profound importance *to animals dependent upon them* because *these levels fall* below their minimum iodine requirements Similarly suitable iodine treatment of such soils is followed by a rise in iodine concentration in the plants to levels sufficient for the requirements of animals but is not accompanied by any improvement in growth or well being of the plants The position with cobalt is identical Plants growing on cobalt deficient soils carry insufficient concentrations of this element to meet the minimum requirements of grazing ruminants but ample for the needs of the plant Iodine and cobalt in fact have not yet been shown to be essential for plant growth At the other end of the scale is an element such as boron which on present evidence is not necessary for animals Plants react to boron deficient soils by a decrease in growth various deficiency

symptoms and lowered internal concentrations of boron A reduction in the levels of tissue boron may restrict the development of the plant but this is of no consequence to animals consuming the affected plants

The position with respect to zinc, manganese and copper is different again These elements are required by both plants and animals but in very different relative proportions On normal soils plants contain levels of zinc and manganese far beyond the minimum requirements of mammals consuming these plants On soils containing subnormal levels of plant available zinc or manganese *the plants themselves show well* defined deficiency syndromes including usually reduced tissue concentrations of the mineral concerned But *they still carry concentrations of these elements sufficient to enable animals to secure their minimum requirements* in so far as these requirements are known or can be deduced In these cases therefore treatment of the deficient soils with zinc or manganese compounds is of great significance to the plants in that it raises their productivity This, however, is of importance from the point of view of the nutrition of animals mainly by virtue of the increased total amounts of essential nutrients other than zinc or manganese *made available to them*

Copper is an example of an element in which the concentration in plants grown on normal soils although very variable is seldom greatly in excess of the requirements of stock consuming the plants In this case soils subnormal in plant available copper generally result in depressed plant growth poor seed production, various deficiency symptoms and usually a lowering of the copper content of the plant tissue to levels below those necessary for the grazing animal to secure its minimum copper requirements Copper application to such soils results therefore, in improved plant growth and health and in improved animal *growth and health mainly through the additional copper* which the treated plants provide

In most copper deficient areas the position is as just described viz that both plants and animals suffer from deficiency and both plants and animals respond to copper fertilization of the soil but there are exceptions referable to the particular plant species that are prevalent in a particular area to the nature of the soil and to the type of animal utilizing the herbage For instance plant species are known that have the capacity to economize on limited supplies of soil copper The low internal concentration in these species though adequate for normal plant growth may be insufficient for the requirements of grazing ruminants Treatment of copper deficient soils carrying such plant species may be of great value to the grazing animal but of no apparent value to

the plant. Further as was pointed out in Chapter 3 horses can be grazed on such copper deficient areas without ill effects because their requirements for this element appear to be appreciably lower than those of sheep and cattle The low concentrations of copper in the herbage under these conditions could therefore limit plant growth but not animal growth

IV The Complexity of Soil Plant-Animal Interrelationships

Very many other examples of soil plant animal interrelationships of great practical significance could be given some of which have been cited in earlier chapters Sufficient has been said however to indicate the extraordinary complexity of these relationships—a complexity which goes far beyond such qualitative and quantitative differences between plants and animals and within plants and animals in their requirements for particular trace minerals as have just been described No mention has been made at this point for instance of trace element interactions such as those between copper and molybdenum which are discussed in some detail in Chapters 3 and 4 These interactions arise in the field primarily as a result of soil differences which determine the relative concentrations of copper and molybdenum in the herbage both directly and by their effect on botanical composition These soil differences influence in the same way the inorganic sulfate content of the herbage with profound effects on molybdenum metabolism and through molybdenum on the copper metabolism of the animals There is little doubt that other interactions of this type will be disclosed by future investigations

A type of soil plant animal interrelationship that was considered in Chapter 11 but that deserves further mention at this point is that which exists in the seleniferous areas of North America The selenium loving *Astragalus* and other species that occur in these areas not only supply highly toxic amounts of selenium directly to animals that consume them but they may supply considerable quantities of this element indirectly to the animals through their capacity to convert forms of soil selenium not available to ordinary crop and pasture plants into forms which are readily absorbed by these plants In this way certain soil types that would otherwise carry herbage relatively harmless to stock are converted to soils from which the herbage can obtain sufficient selenium to be definitely toxic

Perhaps the most remarkable example of soil plant animal interrelationship however is that concerning the incidence of the disease phalaris staggers in sheep and cattle which was described in Chapter 5

This condition is unknown in most areas where the perennial grass *Phalaris tuberosa*, is grown On cobalt deficient, or incipiently cobalt deficient terrain by contrast, the neurotoxic principle that is produced by this plant species induces demyelination and the consequent staggers syndrome in animals unless these animals are regularly dosed with cobalt or the herbage is topdressed with cobalt salts The significant point in relation to the present discussion is that normal soils produce herbage with cobalt concentrations adequate to meet the normal needs of the grazing ruminant plus sufficient to enable them to detoxicate or otherwise handle, the neurotoxic principle in *Phalaris tuberosa* Other soils less satisfactory in cobalt status, are capable of fulfilling the former needs but not the latter, and severely cobalt deficient soils result in the growth of plants in which the concentrations of cobalt are so low that the ruminant confined to them cannot secure enough cobalt to fulfill its needs for growth appetite, or blood formation In none of these soils does it appear that either the growth or the metabolism of the plant is affected

This type of soil plant animal relationship is unique so far as present knowledge goes but it would be extraordinary if other effects of ab normal levels of trace elements in soils on the metabolism of plants and on the capacity of the animal to handle subtle metabolic products as yet unknown were not disclosed as research proceeds Studies of the relation of soil conditions on the one hand and of animal physiology on the other to such constituents of plants consumed by animals present a difficult but intriguing field for research The possibilities of studies of this nature are only now being appreciated

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